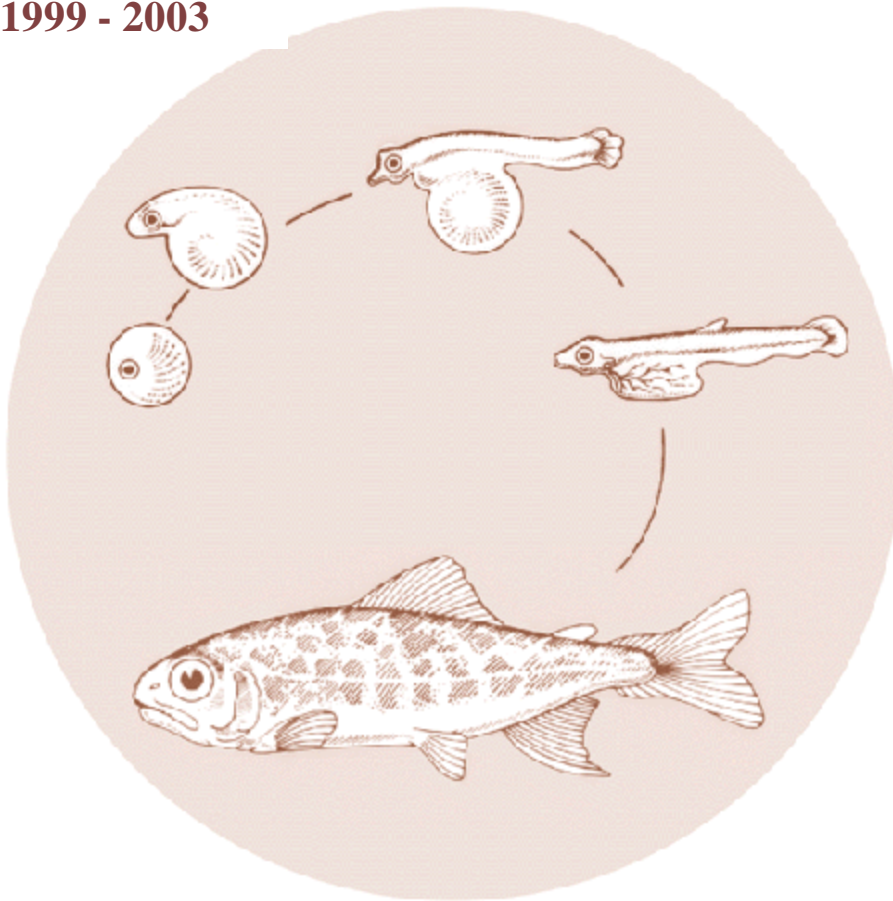


Development of a Natural Rearing System to Improve Supplemental Fish Quality

Progress Report 1999 - 2003



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Bonneville Power Administration
P.O. Box 3621
Portland, Oregon 97208

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**DEVELOPMENT OF A NATURAL REARING SYSTEM TO IMPROVE
SUPPLEMENTAL FISH QUALITY**

**PROGRESS REPORT (PERFORMANCE PERIOD 1 MARCH 1999
THROUGH 28 FEBRUARY 2003)**

Prepared by:

Desmond J. Maynard
Barry A. Berejikian
Thomas A. Flagg
and
Stephen Riley

Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanographic and Atmospheric Administration
Seattle, Washington

Prepared for:

U.S. Department of Energy
Bonneville Power Administration
Environment, Fish and Wildlife
P.O. Box 3621
Portland, OR 97208-3621

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EXECUTIVE SUMMARY

The National Marine Fisheries Service (NMFS), Northwest Fisheries Science Center (NWFS), Resource Enhancement and Utilization Technologies (REUT) Division has been cooperating with the Bonneville Power Administration (BPA) on the Natural Rearing Enhancement System (NATURES) project (Project 199105500) to develop and evaluate new fish culture techniques designed to produce Pacific salmon (*Oncorhynchus spp.*) which are behaviorally, physiologically, and morphologically similar to their wild counterparts.

The major efforts of the NATURES program are directed at developing and evaluating rearing strategies for the production of hatchery salmonids with wild characteristics and increased postrelease survival. In mitigation, enhancement, and conservation hatcheries, salmonids are maintained in a protective environment for less than half their life cycle before being released to survive in the wild environment. Hatchery reared fish may have high migratory and marine mortality, with often considerably less than 1% of the fish surviving to recruit to the fishery or spawning population. This low survival partially stems from traditional fish culture practices failing to prepare fish for survival in their natural environment. NATURES addresses this problem by developing and evaluating more natural fish culture practices that equip fish with the behavioral, physiological, and morphological attributes they need to survive in the migratory corridor and sea. From 1999 to 2002, NATURES researchers have evaluated the effect of exercise, seminatural raceway habitat, and predator avoidance conditioning as tools that can be used to increase the postrelease survival of hatchery salmonids. In addition they have examined how NATURES rearing effects ecological interactions between hatchery and naturally reared salmonids.

NATURES studies conducted from 1992-1994 have shown that the instream survival of chinook salmon *Oncorhynchus tshawytscha* reared in raceways with seminatural habitat composed of gravel substrates, instream structure, and overhead cover may be up to about 50% higher than that of salmon reared in conventional raceways. In 1996, a new experiment was initiated at the Washington Department of Fish and Wildlife (WDFW) Forks Creek Hatchery to determine if seminatural raceway habitat also increases smolt-to-adult survival. Since 1997, fall chinook salmon have been reared from swimup fry to zero-age smolt in 9.75 m long raceways. Each year, half of the raceways have been fitted with seminatural habitat composed of gravel paver substrate, conifer instream structure, and camouflage net overhead cover, while the other half are maintained as conventional controls. The raceways fitted with seminatural raceway habitat can be cleaned with conventional vacuum technology and require only a minor increase in maintenance effort. In most years, chinook salmon reared in seminatural raceway habitat were slightly smaller than controls. The coloration of fish in the two rearing treatments always diverged, with NATURES fish developing a color pattern that seemed more cryptic in the postrelease environment. In laboratory bioassays, fish from seminatural raceway habitat were attacked by hooded mergansers less frequently than conventionally reared chinook salmon. The health of fish reared in seminatural raceway habitat equaled or exceeded that of their conventional controls. The instream survival of smolts reared in seminatural raceway habitat averaged 3.8, 10.0, 24.0, and 1.0% higher than their conventional counterparts in 1997, 1998, 1999, and 2000, respectively. The recovery of coded-wire-tagged salmon released to the sea will

be assessed over the next five years to determine if seminatural raceway habitat rearing produces similar increases in smolt-to-adult survival.

In 2000, a four year study examining the effect of seminatural raceway habitat consisting of gravel-paver substrate, fir tree structure, and camouflage net cover on coho salmon (*O. kisutch*) smolt-to-adult survival was initiated at five WDFW hatcheries, with each facility having one modified (experimental) and one unmodified raceway. The study has successfully generated covers and concrete pavers well suited for use in a large variety of production hatchery rearing vessels (standard raceways, Burrows ponds, and large ponds). Fish are reared in the seminatural raceway habitats for at least the last two months prior to release. In the first two study years, there were no significant differences in fish size or health at release. However, similar to previous NATURES studies, the seminaturally reared fish developed a significantly different base skin coloration as measured by hue, saturation, and intensity. This skin color difference should enhance their crypticity in the natural environment to make them less vulnerable to visually hunting predators (fish, birds, mammals). In future years, the recovery of coded-wire tag data from the fishery and returns to the hatchery will be used to compare the effect of the two rearing strategies on coho salmon smolt-to-adult survival.

In 1999 and 2000, two studies evaluating the effect of exercise on zero-age fall chinook salmon were conducted. In these studies, fish were exercised for no more than two hours a day and the exercise program was suspended during disease outbreaks. Exercised fish in 1999 became longer and heavier than controls fed an equivalent ration. However, in 2000 this growth advantage of exercise failed to reoccur with fish in both rearing treatments growing at similar rates. In both years, the tested exercise protocols successfully reduced cumulative mortality during the primary experimental period. The postrelease survival effect of exercise was evaluated during the summer of 1999 by releasing study fish into a Puget Sound tributary stream. Although some fish at release were observed to better able to hold position against the current than others, the downstream travel time of fish in both treatments was similar. Unexpectedly, significantly more control than exercised fish were recovered at the wier in this postrelease survival evaluation. Unless the exercised fish possessed an enhanced ability to hold position against the current and lacked a migrational urge, their downstream survival must have been diminished by the exercise program. Although exercise slightly increased resistance to hooded merganser attacks, the results were not significantly different.

We also conducted several studies to evaluate the efficacy of chemical conditioning as a predator conditioning tool. We determined whether: 1) populations which evolved in sympatry and allopatry with northern pikeminnows possess innate predator recognition; 2) fright responses to predator odor can be increased by pairing conspecific extract with predator odor; 3) handling affects conditioned responses; 4) acquired predator recognition is retained after transport to a novel environment; and 5) vulnerability to live predators in a stream channel can be improved by chemical conditioning. The experiments were conducted between 1 March and 1 June 2000. Results of these investigations will help guide implementation of anti-predator conditioning in production-scale tests, and maximize the opportunity for successful application.

Ecological interactions between hatchery and wild salmonids, including competition and predation, have been identified as important factors that may negatively affect wild salmonid populations. We performed several experiments comparing naturally-reared juvenile steelhead to conspecifics reared in conventional and NATURES hatchery environments in order to estimate potential effects of hatchery-reared steelhead on wild steelhead in streams. In 2000, we compared fin quality, competitive ability, aggressive behavior, and growth of age-0 steelhead from the same parent population reared in conventional and NATURES (structurally enriched) hatchery environments and a natural stream. Conventionally reared fish had lower mean dorsal fin quality than fish reared in the NATURES environment or the natural stream. Fish reared in the NATURES tanks and the natural stream had similar dominance ranks, and both of these treatments had significantly greater dominance ranks than conventionally reared fish. Dominant fish from the NATURES tanks, conventional tanks and the natural stream differed in the frequency of displays, but not in the frequency of aggressive attacks. Dorsal fin quality did not have a significant effect on dominance. Instantaneous growth rates of fish from conventional and NATURES environments did not differ significantly after approximately one month of rearing in the quasi-natural stream channel, but the growth rate of naturally-reared fish differed depending on whether they were stocked into the channel with NATURES or conventional fish. We suggest that hatchery-reared juveniles released for conservation purposes may acquire increased competitive ability through rearing in enriched habitats, and they may be better able to compete and establish territories in the presence of progeny of non-local hatchery strays.

NATURES studies conducted in 2001 and 2002 focused on differential competitive effects of NATURES and conventionally-reared age-0 steelhead on naturally-reared conspecifics. In 2001, we quantified the feeding rates, agonistic interaction rates, and space use of naturally-reared steelhead at two densities alone and in the presence of NATURES and conventionally-reared steelhead from the same stock in order to examine competitive effects of hatchery-reared fry on wild conspecifics. In 2002, we quantified feeding, agonistic behavior, shelter use, water column position, growth and survival of age-0 steelhead from the three rearing treatments (conventional, NATURES, natural) in the presence and absence of predators (hatchery-reared age-1 steelhead) under laboratory conditions. We also evaluated habitat use, feeding, aggression, territory size, growth and survival of NATURES and conventionally-reared age-0 steelhead that were released into two natural streams. Results of these investigations are currently being analyzed, and will provide insight into how hatchery steelhead fry grown in NATURES and conventional environments may affect wild steelhead in natural streams.

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PREFACE

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DISCLAIMER

Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

INTRODUCTION

The National Marine Fisheries Service (NMFS) has been conducting Natural Rearing Enhancement System (NATURES) research since the early 1990s. NATURES studies have looked at a variety of mechanisms to enhance production of wild-like salmonids from hatcheries. The goal of NATURES research is to develop fish culture techniques that enable hatcheries to produce salmon with more wild-like characteristics and increased postrelease survival. The development of such techniques is called for in the Columbia Basin Fish and Wildlife Program. This document is the draft report for the Supplemental Fish Quality Contract DE-AI79-91BP20651

Over the history of the project, the effects of seminatural raceway habitats, automated underwater feeders, exercise current velocities, live food diets, and predator avoidance training have been investigated. The findings of these studies are reported in an earlier contract report (Maynard et al. 1996a). The current report focuses on research that has been conducted between 1999 and 2002. This includes studies on the effect of exercise on salmon and steelhead trout, effects of predator avoid training, integration of NATURES protocols into production hatcheries, and the study of social behavior of steelhead grown in enriched and conventional environments.

Traditionally, salmon (*Oncorhynchus* spp.) are reared in barren concrete raceways that lack natural substrate, in-stream structure, or overhead cover. The fish are fed in an unnatural manner with artificial feeds mechanically or hand broadcast across the water surface. This traditional approach has increased the egg-to-smolt survival of hatchery-reared fish by an order of magnitude over that experienced by wild-reared salmon. However, once hatchery-reared fish are released into the wild their smolt-to-adult survival is usually much lower than wild-reared salmon.

The reduced postrelease survival of hatchery-reared fish may stem from differences in their behavior and morphology compared to wild-reared salmon. After release, hatchery-reared fish are inefficient foragers and are often found with empty stomachs or stomachs filled with indigestible debris (Miller 1953, Hochachka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986). Their social behavior also differs, with hatchery-reared fish congregating at higher densities, being more aggressive, and displaying less territory fidelity than wild-reared fish (Fenderson et al. 1968, Bachman 1984, Swain and Riddell 1990). In the natural environment this results in hatchery-reared fish spending more time in high-risk aggressive behavior and less time in beneficial foraging behavior than their wild-reared counterparts. Hatchery-reared fish are also more surface oriented than wild-reared salmonids (Mason et al. 1967, Sosiak 1978). This increases their risk of being attacked by avian predators, such as kingfishers (*Ceryle* spp.), which search for fish near the surface. Although some of the differences between wild and hatchery-reared fish are innate (Reisenbichler and McIntyre 1977, Swain and Riddell 1990), many are conditioned and can be modified by altering the hatchery rearing environment. NATURES studies are aimed at developing a more natural salmon culture environment to prevent the development of these unnatural attributes in hatchery-reared fish.

NATURES fish culture practices are already producing salmon with up to about 50% higher in-stream survival than conventionally-reared fish (Maynard et al. 1996b). When these

techniques are incorporated into production releases, they should also translate into increased smolt-to-adult survival. Conservation and supplementation programs can use NATURES-reared salmonids to rebuild stocks currently listed as endangered and threatened into healthy self-sustaining runs more rapidly than traditional programs. Traditional production programs can also use high-survival NATURES-reared fish to reduce their impact on wild populations, while still meeting their adult mitigation goals.

The following is a list of NATURES related articles published during the project:

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Section 1

EFFECTS OF SEMINATURAL HABITAT ON CHINOOK SALMON REARED AT THE WDFW FORKS CREEK HATCHERY, 1997-2000

by

**Desmond J. Maynard, Gail C. McDowell¹, Thomas A. Flagg, Chuck Johnson²,
Barbara Cairns³, Glen A. Snell, John Colt, Anita L. LaRae¹, James L. Hackett,
George Britter², Brodie Smith³, Conrad V. W. Mahnken, and Robert N. Iwamoto**

Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
Manchester Research Station
P.O. Box 130
Manchester, Washington 98353

¹Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon 97027

²Washington Department of Fish and Wildlife
600 Capitol Way North
Olympia, Washington 98501-1091

³Long Live the Kings
P.O. Box 21605
Seattle, Washington 98111

Introduction

The National Marine Fisheries Service (NMFS) has been developing a Natural Rearing Enhancement System (NATURES) consisting of seminatural raceway habitat, live food diets, exercise systems, predator avoidance training, underwater feed delivery systems, etc. since 1990 (Maynard et al. 1995; 1996 a,b,c,d). Seminatural raceway habitat composed of gravel substrates, instream structure, and overhead cover has been used to produce a more natural hatchery environment. In past studies (Maynard et al. 1996c) the instream survival of chinook salmon reared in this seminatural raceway habitat was up to about 50% higher than for fish reared in conventional raceways. However, it is unknown if this increase in instream survival leads to increased smolt-to-adult survival. Therefore NMFS, Long Live the Kings (LLTK), Washington Department of Fish and Wildlife (WDFW), Bonneville Power Administration (BPA), and the Weyerhaeuser Corporation initiated a multiyear production-scale test to determine if rearing fall chinook salmon in seminatural raceway habitat also increases smolt-to-adult survival. The study was primarily funded by NMFS, WDFW, and LLTK. BPA participation was focused on coordinating information transfer of NATURES variables developed under BPA funding to the Forks Creek experiment. This report focuses on the progress of the study through 2001.

Methods

The experimental facility was developed in 1996 with the installation of eight fiberglass raceways on a concrete pad at the WDFW Forks Creek Hatchery. Forks Creek is a tributary of the Willapa River. The raceways (9.75-m long \times 2.44-m wide \times 1.24-m high) maintained a water depth of 0.80 m. The interior of all eight raceways was originally dark gray (10 on the Kodak gray scale). In 1998, the interior of the control raceways was changed to a lighter shade of gray (2 on Kodak gray scale) to resemble the lighter color of concrete.

The control raceways were left unmodified for the most part. In all 4 years of operation the top of each control raceway was covered with nine aluminum-frame panels (1.96 \times 2.64 m) to prevent avian predation in the control tanks. The panels were fitted with white nylon netting (0.6 \times 0.6-cm mesh) to prevent small predators, such as dippers (*Cinclus mexicanus*), from entering.

The experimental raceways were fitted with resin rock paver substrate, a submerged Douglas fir tree structure, and covered with (U.S. military) camouflage netting. The resin rock pavers were tiles (0.6 \times 0.6 m) fabricated with epoxy resin and river gravel (22 - 32 mm diameter). The river gravel was carefully selected to match the color of the sand and gravel found in Forks Creek. All needles were removed from each fir tree before weighting with rebar. Five trees were suspended from a single horizontal cable running the length of each raceway, preventing them from touching the bottom. The trees were suspended from the horizontal cable by vertical cables attached to each end of their trunk. Each free cable end was then attached to the horizontal cable with an interlocking spring snap so the trees could be easily moved or removed during cleaning. A single layer of camouflage net was hung along both ends of the cover so that, when the panels were in place, there was a 40% covered area running along each side of the raceway top with the middle 20% camouflage free.

1997 Activities

In 1997, four experimental raceways were used for the study. On 24 February 1997, identical weights (approximately 54,000 fish) of fall chinook salmon swim-up fry were ponded into each raceway. The fish were reared following standard WDFW fish culture practices (Michak 1997). Each raceway was cleaned at least once every week with a commercial swimming-pool vacuum. All mortalities were counted and removed.

Every month a sample of 100 fish was removed from each raceway, weighed (to the nearest 0.001 g), measured (fork length to the nearest 1 mm), and means compared with *t*-tests. At least 30 fish in each sample were photographed with 400 ASA color slide film using a Nikon 8000S single lens reflex camera equipped with a micro lens (60 mm) and circular polarizing filter. The camera was mounted on a photographic light stand equipped with two quartz halogen lamps (300 W). The light was filtered through photographic gel to simulate daylight.

Before being photographed, the fish were anesthetized in tricaine methane-sulfonate (MS 222) solution in black dishpans, and then placed individually on a clear acrylic angled stand over a standardized blue background. The fish were photographed at least twice.

Each photograph was mounted in a standard plastic slide mount. This enabled it to be placed on a PVC plate (with the center drilled out) attached to the stage of a stereoscopic binocular microscope. A fiber-optic light illuminated the slide from below. The image was then picked up by a Hyper HAD RGB color video-camera. The video-image was then captured and processed by image analysis software. For skin color analysis, a rectangular section of the caudal fin was examined on each fish for hue, intensity, and saturation values. These values were compared with *t*-tests.

Over a 2-week period in late April and early May 1997, approximately 51,000 fish in each raceway were coded-wire tagged and adipose-fin clipped for subsequent evaluation of survival (smolt-to-adult). The fish in each raceway received a unique batch code. These fish were reared on and then released from the hatchery in two paired releases on 7 and 9 June. The regional database of the Pacific States Marine Fisheries Commission (PSMFC) will be used to compare the recruitment to the fishery and relative survival to spawning of these releases of conventionally- and seminaturally-reared fish from 2003 on.

On 4-6 June 1997, a subsample of 750 fish from each raceway was PIT tagged for an instream survival and migration speed evaluation. The fish were returned to their raceway and allowed to recover from the effects of tagging. Later, they were transported in a white fiberglass tank to the upper watershed of Forks Creek (location 46° 30' 51" N and 123° 32' 8" W) where they were released in paired groups. Releases were made on 16 and 23 June 1997. Each paired group consisted of all the PIT-tagged fish in one conventional and one seminatural raceway. The fish migrated downstream and were recaptured at a weir located at the hatchery (46° 33' 26" N and 123° 35' 46" W), and their PIT tag code interrogated. After all the codes were recorded, the fish were released below the weir to continue their migration to sea. Numbers recaptured were compared with 2 × 2 contingency table analysis, and migration speed compared with ANOVA.

A subsample of 30 fish was removed from each raceway on 4-6 June 1997 for pathological analysis. These fish were euthanized in MS 222 and then dissected for evaluation.

The condition of the spleen was observed and subjectively rated. The posterior third of the kidney was removed and examined for the presence of *Renibacterium salmoninarum*, the causative agent of BKD, and the fluke *Nanophyetus salmincola*. Portions of the kidney were streaked on agar plates, incubated at 20°C, and examined after 24 hours for evidence of bacterial pathogens. *N. salmincola* cyst counts were analyzed with a *t*-test.

1998 Activities

In 1998, the number of raceways per treatment was increased from two to three. The inside walls and bottom of the control raceways were lined with a light gray fiberglass reinforced panel (2 on Kodak gray scale) to resemble the light gray color of concrete (1 to 2 on Kodak gray scale). The original resin rock pavers were replaced with new pavers made of resin that did not turn white when submersed in water. Except for these modifications the control and seminatural raceways remained the same as in 1997.

The second experiment was initiated on 27 January 1998. An equal weight of fall chinook swimup fry (approximately 37,035 fish) was ponded into each of the experimental raceways. The fish were again reared following standard WDFW procedures. Sampling for growth and coloration followed identical methods to those used in 1997, beginning 2 February 1998. The number of fish photographed was maintained at 60 per treatment but reduced to 20 per raceway.

Over a 2-week period in April 1998, at least 33,500 fish in each raceway were tagged and fin clipped for the evaluation of smolt-to-adult survival. These fish were then reared until 1 June 1998, when they were released from the hatchery below the Forks Creek weir. The PSMFC regional database will be used to compare the recruitment to the fishery and relative survival to spawning of fish released from conventional and seminatural rearing habitats from 2003 on.

In spring 1998, the vertical position of the fish in each raceway was recorded using a grid and an underwater video system. The grid had four cells stacked vertically between the surface and bottom of the tank. Data were recorded on videotape (8 mm) for subsequent analysis. A video cassette recorder was then used to play the images back into a Pentium-II computer system. A frame grabber and image analysis software were then used to freeze the image of fish against the grid at 5-minute intervals. A total of 10 intervals were sampled from each individual raceway taping session. The number of fish observed in each grid section was then counted and converted to percentages to compare the in-culture depth behavior of fish in conventional and seminatural rearing habitats.

On 2-3 June 1998, 30 fish from each raceway were sacrificed for pathological evaluation, which differed slightly from that carried out in 1997. The fish were first euthanized in MS 222. The fin condition was assessed, and fish with eroded or split fins were scored. The coelomic cavity was opened and the condition of major internal organs assessed. These results were compared with 2×2 contingency table analysis. The kidney was then sampled and evaluated as previously described for 1997.

On 2-3 June 1998, 500 fish from each raceway were PIT tagged for instream survival and migration speed evaluations. These fish were transported and released in three paired releases at

the same upper watershed location as in 1997. Paired releases were made on 10, 17, and 24 June 1998. Survival was compared with 2×2 contingency table analysis and travel time with ANOVA.

1999 Activities

In 1999, the study was again conducted by rearing fish in three control raceways and three seminatural raceways. The fiberglass-reinforced paneling installed on the control raceways in 1998 was replaced with a more durable gelcoat finish. Other structural repairs were made to the raceways at this time to ensure they last the duration of the experiment. These modifications and repairs were the only changes made in the experimental variables from previous years.

The experiment was initiated on 5 February 1999, with approximately 36,519 fall chinook salmon swimup fry being ponded into each raceway. Except for the experimental variables, these fish were reared following the same standard WDFW salmon culture practices used in the two previous years. The raceways were vacuumed weekly and in-culture mortalities counted and removed when observed.

Beginning on 12 February 1999, a sample of 100 fish from each raceway were weighed and measured every month. During these monthly sampling periods, photographs of 20 fish in each raceway were taken and analyzed as previously described.

Over a two-week period in April 1999, 36,767 or more fish in each raceway were coded-wire tagged and adipose-fin clipped for the smolt-to-adult survival evaluation. These fish were then reared until 4 June 1999, when they were released from the hatchery below the Forks Creek weir. The Pacific Northwest regional coded-wire tag (CWT) database will be consulted annually from fall 2001 to 2005 to compare the recruitment to the fishery and relative survival to spawning of fish reared conventionally versus seminaturally.

In the spring of 1999 the vertical position of the fish in each raceway was recorded with an underwater 8mm standard video system. In these sessions all six raceways were videotaped in a single day of taping. Methods were identical to those used in 1998.

On 2 June 1999, a subsample of 30 fish from each raceway was euthanized in MS 222 for pathological evaluation. These fish were examined using a standard condition profile (Goede Index; Goede and Barton 1990). The external condition of the fish was first assessed according to the Goede Index. The tail was severed and blood collected in a heparinized microhematocrit tube for centrifuge and reading of blood parameters. The coelomic cavity was opened and the condition of major internal organs assessed. These results were compared with 2×2 contingency table analysis, and t-test for blood parameters. Finally, the kidney was sampled for presence of bacterial kidney disease, and streaks from their kidney tissues were plated to evaluate pathogen presence.

On 8-9 June 1999, a systematic subsample of 490 fish from each raceway was PIT tagged for an instream smolt-to-smolt survival evaluation. As in previous years, these fish were transported and released in three paired releases into the upper watershed of Forks Creek on 17 June, 24 June, and 1 July 1999. The downstream migrants were recaptured at a weir located at

the hatchery. Instream survival was compared with 2×2 contingency table analysis, and migration speed compared with ANOVA.

2000 Activities

The final rearing and release year of the experiment was initiated on 8 February 2000. An equal weight of fall chinook swimup fry (approximately 36,615 fish) was ponded into each of the experimental raceways. The fish were again reared following standard WDFW procedures. Beginning 18 February 2000, samples of 100 fish from each raceway were weighed and measured every month as before, and means analyzed with *t*-tests. The number of fish photographed was maintained at 60 per treatment.

Over a 2-week period in April 2000, at least 35,500 fish in each raceway were coded-wire tagged and fin clipped for the evaluation of smolt-to-adult survival. These fish were then reared until 31 May 2000, when they were released from the hatchery below the Forks Creek weir. From fall 2003 on, the PSMFC database will be consulted to compare the recruitment to the fishery and relative survival to spawning of fish from the two rearing treatments.

In spring 2000, the vertical position of the fish in each raceway was recorded using a grid and an underwater video system. The grid had four cells stacked vertically between the surface and bottom of the tank. Data were recorded on 8-mm videotape for subsequent analysis. Data were analyzed in the same fashion as in 1998 and 1999.

Throughout rearing, measurements were taken to monitor physical and chemical differences between seminatural and conventional rearing units. Information was collected on dissolved oxygen, total gas pressure, temperature, total ammonia nitrogen, un-ionized ammonia nitrogen, pH, suspended solids, and light levels.

Dissolved oxygen, total gas pressure, and temperature were measured on-site, in a single seminatural and conventional tank, on five different dates (5, 12 and 26 April, 11 and 24 May 2000). Dissolved oxygen (DO) was measured using a YSI Model 57, serial number 89K009809, using method 4500-0G (Clesceri et al. 1998). The meter was calibrated with a YSI calibration chamber using the saturation values developed by Benson and Krause (1980). The probe was placed in the raceway inflow (influent) and between the external and internal standpipes (effluent). Water temperatures were measured using a precision glass thermometer with calibration traceable to NIST certified standards, with measurements taken at the same locations as for dissolved oxygen. Barometric pressure and total gas pressure were measured with a Sweeney Saturometer, Model DS1-A, serial number 074, using standard method 2810 (Clesceri et al. 1998). Measurements were taken on the bottom of the raceway, at the head end and in front of the effluent screen.

Water samples for ammonia and pH were collected in 300 mL BOD bottles with ground-glass stoppers on the same five dates as DO, total gas pressure, and temperature. Samples for suspended solids were collected in either 300-mL BOD bottles or 500-mL bottles with threaded caps. Bottles were rinsed twice with sample water prior to filling. Morning and afternoon water samples were taken from the raceway inflow (influent) and the overflow discharge (effluent) of all six experimental tanks. An additional sample was collected from a single production

raceway, within 2 inches of the overflow weir near the water surface, for comparative purposes. Samples were transported back to the lab and kept at 4°C and in darkness until the analysis was completed. The ammonia and pH analysis was completed within 24 hours of sampling and suspended solids within 48 hours of sampling. Total suspended solids were measured using the standard method (2540D; Clesceri et al. 1998). Initially, suspended solids samples were going to be run on 300-mL samples. Because of analytical errors due to very low suspended solids concentrations, it was necessary to increase the sample volume to 1000 mL or larger. This required sample size limited the number of samples that could be run because of limited availability of large bottles. Effluent samples were run in duplicate after the suspended solids sampling was terminated.

Total ammonia nitrogen was measured using the manual phenate method (4500-NH₃; Clesceri et al. 1998). Un-ionized ammonia was computed using the pKa values developed by Emerson et al. (1975). pKa values were corrected for non-ideal conditions using the Debye-Huckel equation (Stumm and Morgan 1981, Truesdale and Jones 1974). The ionic strength of the water was estimated from conductivity (Snoeyink and Jenkins 1980).

Light levels were measured as 30-second averages at various locations within the tanks on 14 June 2000. The tanks were divided into 27 locations (see Fig. 1 for the location key). In addition, the light levels could be modified by removing the covers and adjusting the location of the trees in the seminatural tanks. Light levels were measured with the LI-COR LI-193SA spherical quantum sensor. This sensor measures energy in 400 to 700 nm wavelengths (Federer and Tanner 1966). The LI-193SA sensor is designed for underwater operations and is enclosed in an acrylic diffuser that measures light from all directions. This measurement is referred to as Photosynthetic Photon Flux Fluence Rate (PPFFR) or Quantum Scalar Irradiance. The units of PPFFR are micromoles of quanta per second per square meter ($\mu\text{mol}/\text{s}\cdot\text{m}^2$).

Light intensity can also be measured as illuminance in lux. Illuminance measures visible radiation with a sensor having a spectral response equal to the average human eye. This measurement is appropriate when the human eye is the primary receiver, such as illumination of work areas. A measurement of illuminance could not be directly applied to fish because of significant differences in spectral response between the two species. In addition, underwater photometric sensors are not readily available. For natural sunlight, it is likely that there is a significant correlation between PAR and PPFFR.

To compare the differences in light levels between the seminatural and conventional tanks, two LI-193SA were used. The sensors were mounted in a clear plastic frame assembly. The center of the sensor was elevated 8.5 inches to allow attachment of an underwater connector to the sensor. During light measurements, both sensors were lowered to the bottom of a seminatural or conventional raceway. The two LI-193SA sensors were connected to a LI-1400 data logger.

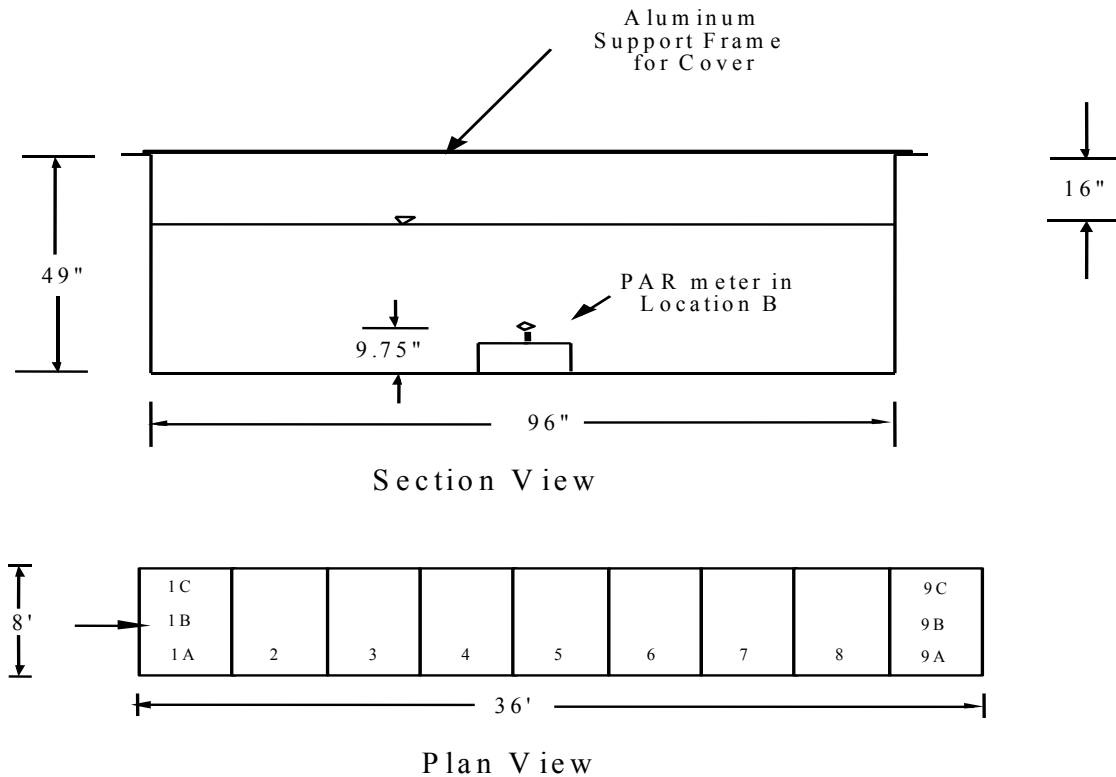


Figure 1. Schematic of experimental tanks with location of light measurements.

On 7-8 June 2000, 30 fish from each raceway were sacrificed for pathological evaluation, using the same technique as in 1999. The Goede Index was again utilized for general condition and blood parameters, and the kidney was then sampled and evaluated as previously described for 1999.

On 13-14 June 2000, 500 fish from each raceway were PIT tagged for instream survival evaluations. These fish were transported and released in three paired releases at the same upper watershed location as in previous years. Paired releases were made on 22 June, 29 June, and 6 July 2000. Survival was again compared with 2×2 contingency table analysis, and migration time compared with ANOVA.

Beginning in May 2000, fish were size-matched at Forks Creek Hatchery and transported to Manchester for predation bioassay experiments. A single length-class was determined for selection (i.e., 80 mm). Equal numbers of fish belonging to this size class were selected from each raceway. Fish were transported in two hauling containers, one per treatment, from the Forks Creek Hatchery to the NMFS Manchester Research Station, once weekly. Fish held at Manchester were held in tanks of similar coloration to those at Forks Creek, and with pavers, trees, and cover in the seminatural treatment, as in their original rearing environment at Forks Creek. Fish were not fed during their tenure at Manchester. Multiple trials were run each day, every day until the supply of fish was exhausted. By doing this, fish were held in Manchester tanks for 1 to 12 days (70% of trials were run within 5 days of transport), ensuring that cryptic coloration was not greatly affected by their holding tanks.

Trials were begun the day after fish were transported to Manchester. Fish were not tagged externally, so that no visibility disadvantage could be conferred. The binary coded-wire tags were used postmortem to determine rearing treatment. Trials were conducted in two 5,947-L raceways located in a fenced enclosure. One raceway was left barren, and kept clean of algae or sediment (“barren” arena). The other raceway was allowed to grow algae on the sides, and its bottom was covered with rock, which was swept up along the edges of the tank to resemble a streambed (“stream” arena). Trials were alternated between the two arenas.

Each trial began by removing 10 fish from the seminatural tank and 10 fish from the conventional tank, and placing them in a single bucket. This bucket was carried into the test arena, where fish were released into the downstream end (above the drain sump). Within five minutes of fish being placed in the test arena, a single hooded merganser (*Lophodytes cucullatus*) was allowed access to the arena for 20 minutes. After twenty minutes, the bird was herded back out of the arena, the tank drained, and the remaining fish removed. Fish were sorted according to condition (killed, live scarred, or live unscarred), euthanized in MS-222, put in labeled bags, and frozen for later analysis.

Trials were continued until all fish were used. One trial had only 8 seminaturally-reared and 8 conventionally-reared fish because of mortality in the holding tanks. When one supply of fish was exhausted, more were sorted and transported from Forks Creek. This process was repeated until a total of 58 trials were conducted, 29 per arena.

Recoveries from a single trial were removed from the freezer and allowed to partially thaw. The heads were removed from each fish, and then scanned for the presence of a coded-wire tag (CWT). Tags were cut out of the heads and placed in labeled vials of alcohol (one for each “condition” – killed, scarred, and unscarred). Any fish head, which scanned negative for a CWT, was individually labeled and returned to the freezer for later quality control. The three vials for the trial were then taken for CWT reading.

Tags were read using a stereoscopic binocular dissecting microscope. A fiber-optic light illuminated the CWT from above. The image was then picked up by a Hyper HAD RGB color video camera, and viewed on a video monitor. Each CWT was read and saved.

When all tags had been read from a single trial, a process of elimination was used to determine the treatment of any fish eaten. For example, if 10 seminaturally-reared fish and 9 conventionally-reared fish were recovered, then the eaten fish was from the conventional treatment. Contingency table analysis (2×2) was used to analyze data.

2001 Activities

In 2001, preliminary data was collected to compare the recruitment to the fishery and relative survival to spawning through 2000 of fish from the two rearing treatments. WDFW maintains a database with all of the CWT releases and recoveries they handled. This is a subset of the PSMFC Regional Mark Database. The WDFW database was queried in the summer of 2001 and release data compared against records kept by NMFS researchers during the four rearing and release years (1997-2000) of the Forks Creek NATURES study. All recoveries of fish from the CWT release groups (hatchery rack, ocean sport, and coastal gillnet) reported to

WDFW through 2000 have been collected for smolt-to-adult survival comparisons. In addition to the WDFW database, the PSMFC regional database was queried to retrieve CWT recoveries from agencies other than WDFW. Total numbers of CWT recoveries were used for the 2×2 contingency table analysis of smolt-to-adult survival.

Results

General

A landslide in the Forks Creek watershed upstream of the hatchery intake occurred in early 1997. This resulted in heavy siltation during the first 6 weeks of rearing. Although the raceways were cleaned daily with a vacuum, thick sediment built up each day throughout this period. By late spring much of the sediment had been washed out, and the bottoms of the conventional raceways were clean of silt. By 1998, the bottoms of the conventional raceways were easily cleaned except when high water levels exposed new parts of the landslide.

In 1997, the original pavers turned white after several days in water. They also crumbled when taken out of the raceways. The resin rock pavers used in 1998 were a distinct improvement with the resin remaining transparent.

In 1997, it was necessary to remove the weir temporarily before PIT-tag recoveries were complete due to heavy flooding. The weir was modified for the 1998 field trials and was not removed during the recovery period in either 1998, 1999, or 2000.

The resin rock pavers markedly reduced vacuuming time compared with that for cleaning loose gravel. Improvements in the vacuum heads and larger wheels to roll over the rocks enabled the seminatural raceways to be cleaned quickly. However, there is still room for improvement as it still takes longer to vacuum raceways with resin rock pavers than conventional smooth bottoms. In general it took three passes with a seine net to catch almost every fish in a seminatural raceway. It took two passes to net almost all the fish in a conventional raceway.

The five fir trees suspended from a wire into each raceway were relatively easy to maintain and work around. The system enabled trees to be unclipped and rapidly removed when it was time to crowd the fish for sampling or removal. Although some branches were lost each season, the trees lasted for all four experimental rearing years.

The camouflage net covers were also easy to work around. The covers were lifted and one side hung from a wire to provide easy access for feeding, vacuuming, or removing mortalities. For seining operations the covers were easily removed and temporarily stored next to the tank. The standing wall tanks and covers successfully eliminated all avian and mammalian predation from the study raceways, even though birds and otters were seen to prey on fish in the uncovered production raceways and ponds at Forks Creek Hatchery.

Growth

In 1997, the seminaturally-reared fish were significantly shorter and weighed less than

conventionally-reared fish at ponding (Figs. 2 and 3). This difference in size had disappeared by the second sampling period. By the third sampling period the conventionally-reared fish had again become significantly longer and heavier than the seminaturally-reared fish. Even after reducing the ration fed to conventionally-reared fish, they remained slightly larger than seminaturally-reared fish at the last sampling period.

In 1998, there was no significant difference in the length or weight of fish at either the first or second sampling periods (Figs. 4 and 5). By the third sampling period, the seminaturally-reared fish weighed significantly less, but were not shorter, than the conventionally-reared fish. In the fourth sampling period in April 1998, the seminaturally-reared fish were both significantly shorter and weighed less than their conventionally-reared counterparts. Although feed was withheld from conventionally-reared fish to allow the seminaturally-reared fish an opportunity to catch up, the conventionally-reared fish were still slightly larger than the seminaturally-reared fish at the end of May 1998.

In 1999, the mean lengths and weights (Figs. 6 and 7) of the two rearing types were similar, with the seminaturally-reared fish usually being slightly, but not statistically significantly, smaller and lighter than the controls. Although there was no significant difference at the last subsampling, seminaturally-reared fish were smaller than the controls.

In 2000, there was no significant difference in the length or weight of fish in the first eleven weeks of rearing (Figs. 8 and 9). By the fifth sampling period, the seminaturally-reared fish weighed significantly less, but were not shorter, than the conventionally-reared fish. In the sampling of 17 May 2000 the seminaturally-reared fish were both significantly shorter and weighed less than their conventionally-reared counterparts. Withholding feed from the conventionally-reared fish allowed the seminaturally-reared fish an opportunity to catch up, so that fish in both treatments were of similar size at the end of May 2000.

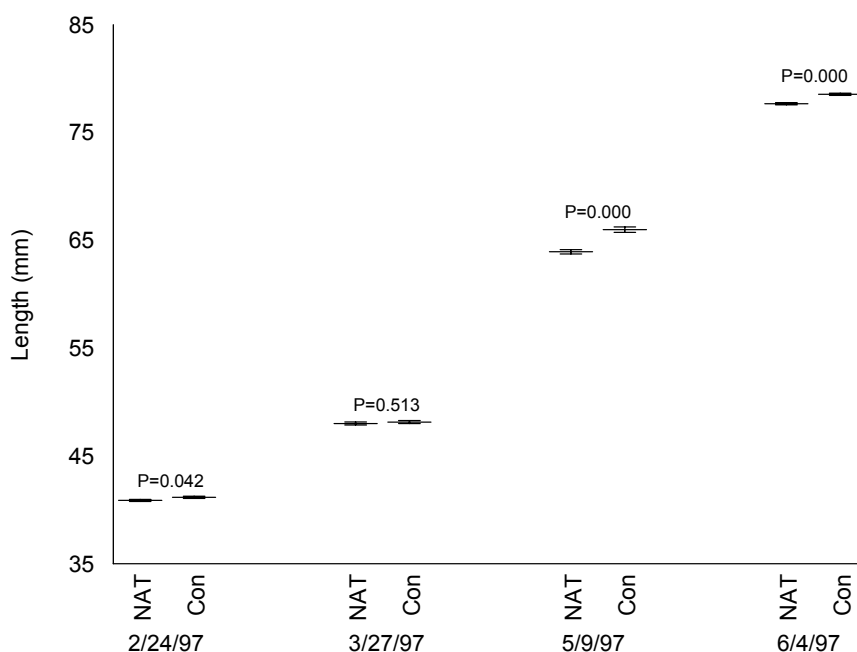


Figure 2. Mean lengths (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 200) or conventional (con, n = 200) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

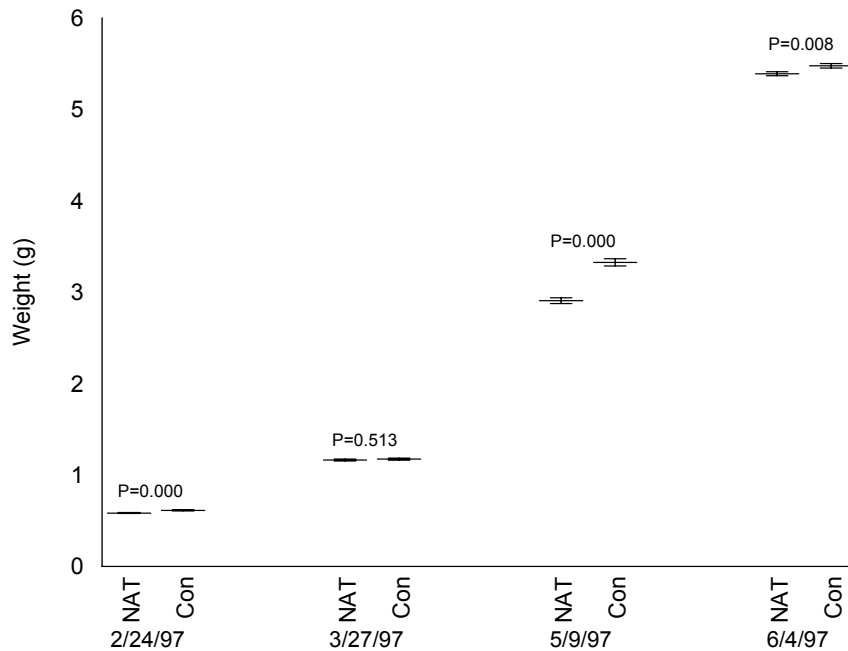


Figure 3. Mean weights (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 200) or conventional (con, n = 200) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

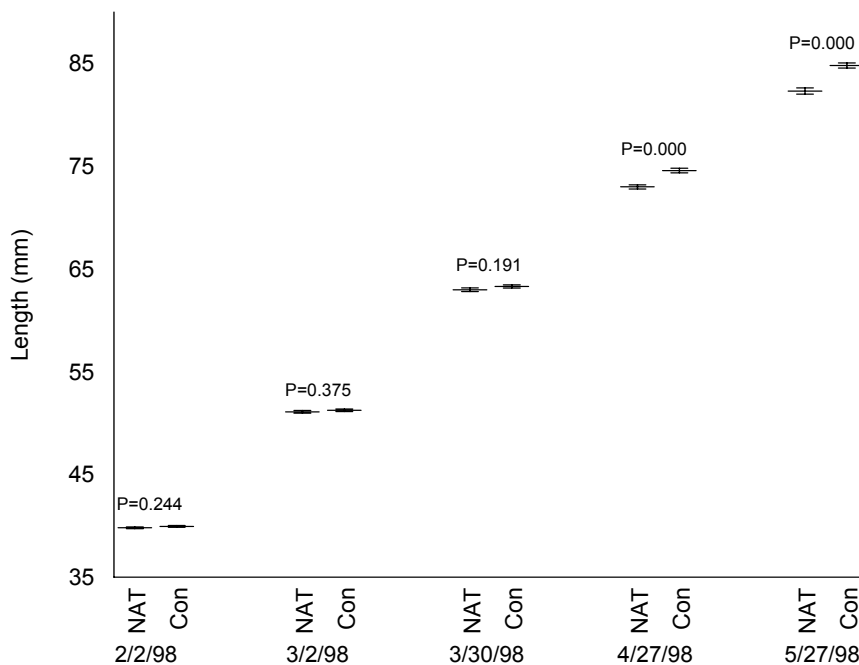


Figure 4. Mean lengths (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

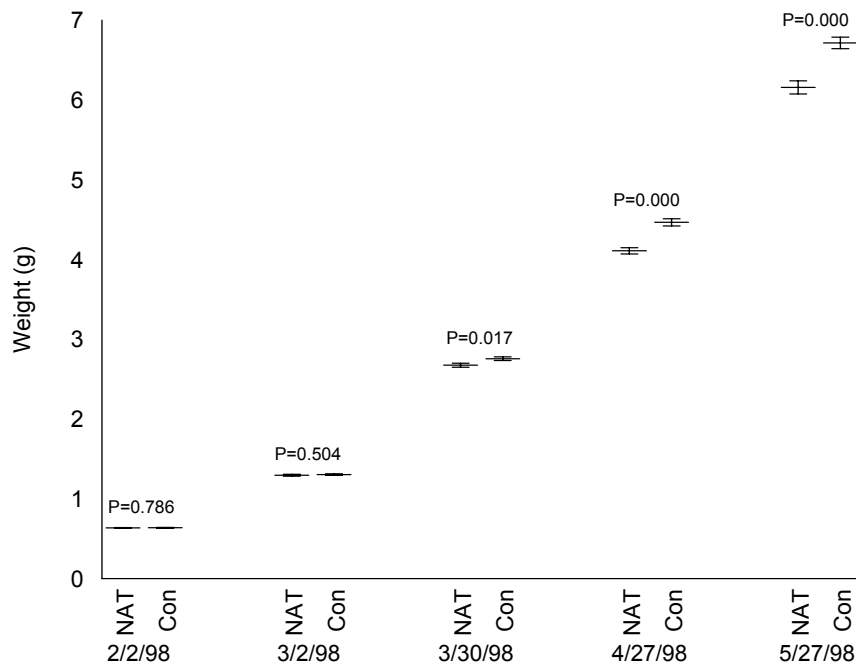


Figure 5. Mean weights (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

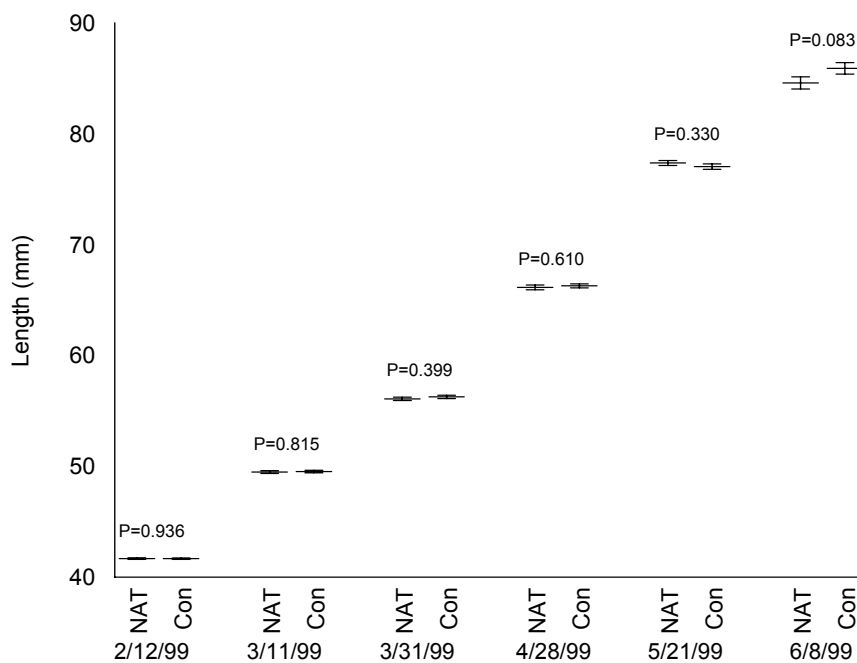


Figure 6. Mean lengths (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 1999. P values are based on *t*-tests.

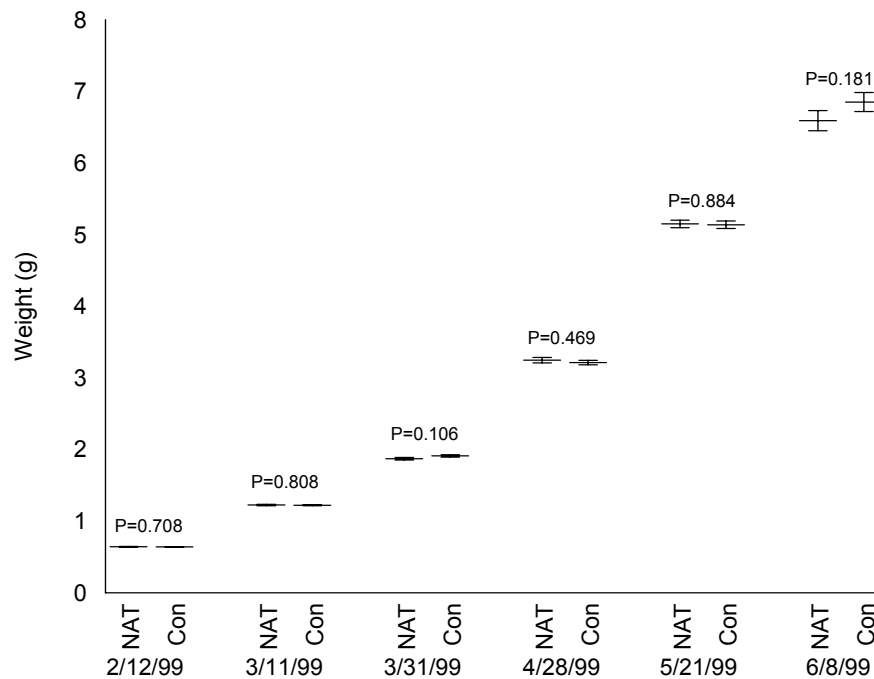


Figure 7. Mean weights (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 1999. P values are based on *t*-tests.

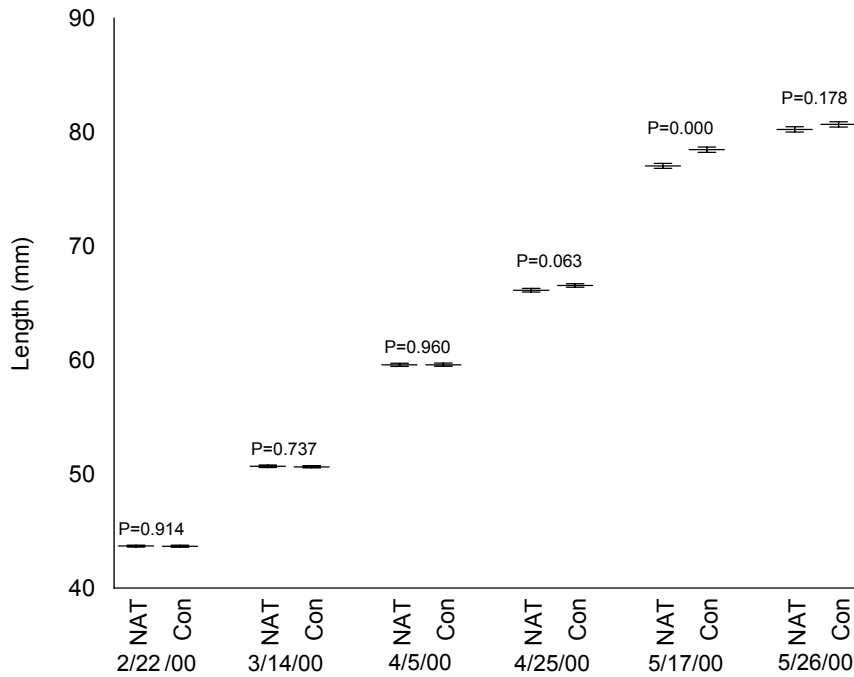


Figure 8. Mean lengths (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 2000. P values are based on *t*-tests.

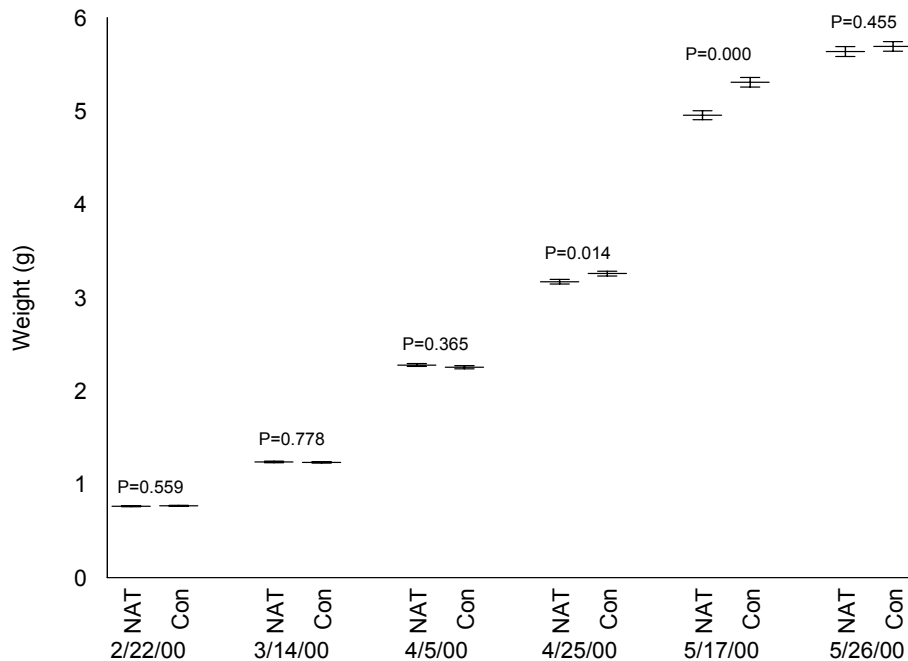


Figure 9. Mean weights (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 300) or conventional (con, n = 300) raceways at Forks Creek Hatchery in 2000. P values are based on *t*-tests.

Coloration

In 1997, fish were photographed the day they were ponded into the two rearing treatments. The skin color values for both treatments were similar at ponding (Figs. 10, 11, and 12)¹. Statistically significant differences did not develop between the two treatments until the last sampling period in June 1997. Only two (hue and intensity) of the three color axes were significantly different from one another just prior to release. Subjectively, the seminaturally-reared fish appeared darker than the conventionally-reared fish. The caudal and pectoral fins of control fish were translucent, while the caudal fin of seminatural habitat fish were brown. There also appeared to be much more chromatophore development in the ventral region of fish reared in seminatural rather than conventional raceways.

In 1998, photographs were taken when the fish had spent a week in their respective rearing treatments. This time was found to be insufficient for the fish to develop skin color differences that were statistically different (Figs. 13, 14, and 15). By March, the skin color differences were statistically different and these differences continued through the last sampling period in May 1998. Although all three color values were statistically different at intermediate sampling periods, only two of the three color axes (hue and intensity) were statistically different at the final sampling. In 1998, the subjective color differences between conventionally- and seminaturally-reared salmon appeared to be greater than in 1997. Again, seminaturally-reared fish had brown-tinged fins and more chromatophore development in the ventral region than conventionally-reared salmon.

In 1999, within a week of ponding significant differences in skin coloration had already developed. The hue and saturation values for seminatural habitat and control fish were almost significantly different on this first sampling a week after ponding (Figs. 16 and 17). The intensity values were significantly different at this time (Fig. 18). For the most part, these color differences remained for all three color axis components throughout rearing. Only intensity values became nonsignificantly different in the March sampling. As in the previous year, subjectively the seminaturally-reared fish appeared to have brown colored fins and more melanophore development in the ventral region.

In 2000, photographs were taken when the fish had spent ten days in their respective rearing treatments. Once again, both hue and saturation were still similar, but intensity differed significantly between treatments (Figs. 19, 20, and 21). By April all three color axes had statistically significant differences. Although all three color values were statistically different at intermediate sampling periods, only two of the three color axes (saturation and intensity) were statistically different at the final sampling. Again, seminaturally-reared fish had brown-tinged fins and more chromatophore development in the ventral region than conventionally-reared salmon.

¹ The reader should be cautioned to make comparisons only between treatment values for hue, saturation, and intensity (i.e., only look at relative differences between treatments). Values should not be compared between sampling dates, as sources for film and processing, and developing times were not always consistent from one sample to the next.

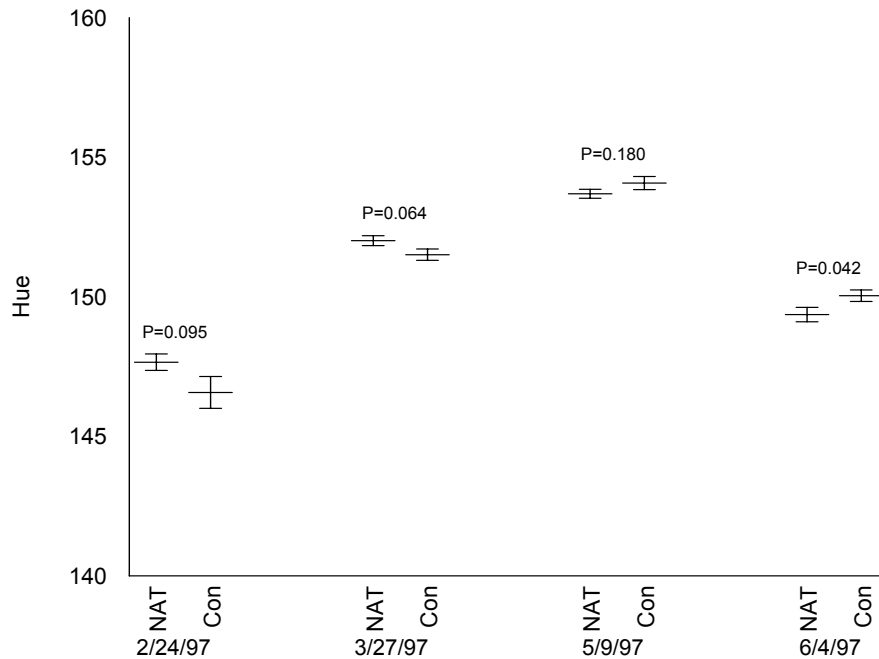


Figure 10. Mean hue values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, $n = 60$) or conventional (con, $n = 60$) raceways at Forks Creek Hatchery in 1997. P values are based on t -tests.

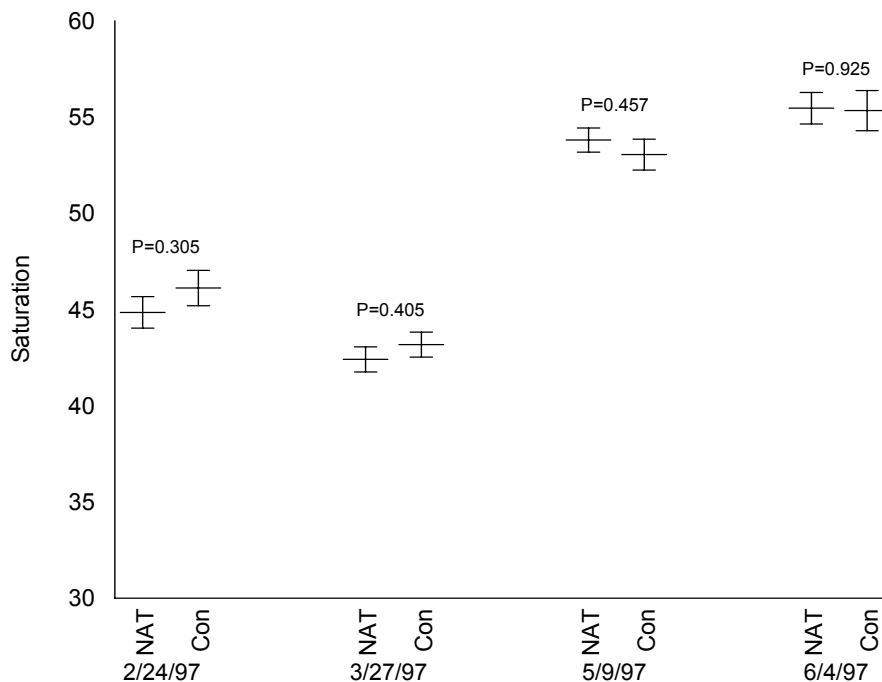


Figure 11. Mean saturation values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, $n = 60$) or conventional (con, $n = 60$) raceways at Forks Creek Hatchery in 1997. P values are based on t -tests.

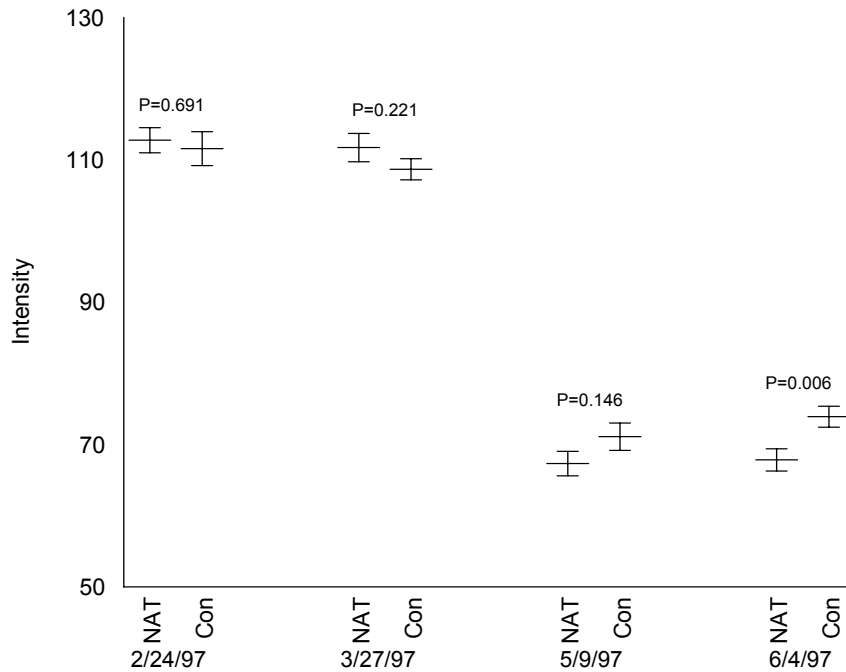


Figure 12. Mean intensity values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

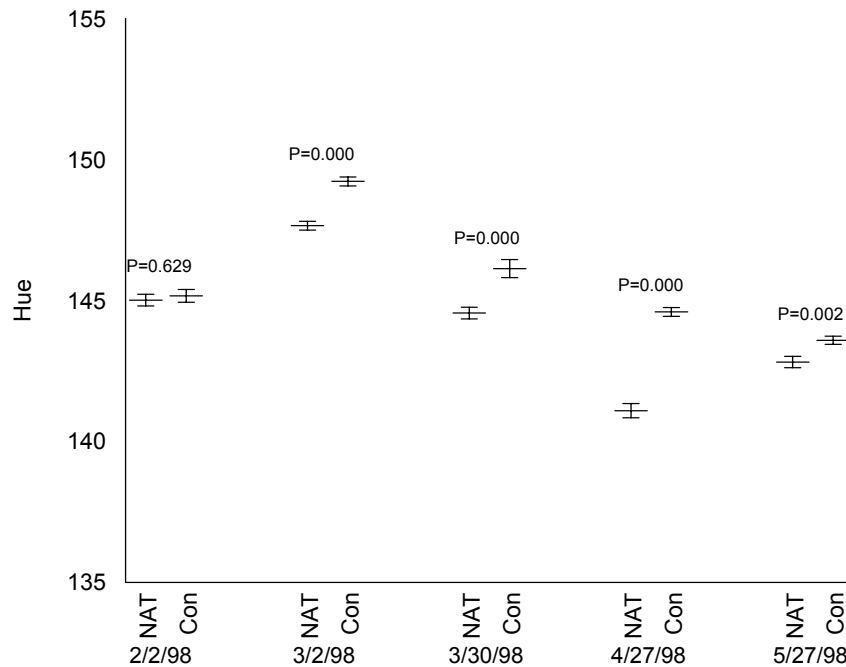


Figure 13. Mean hue values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

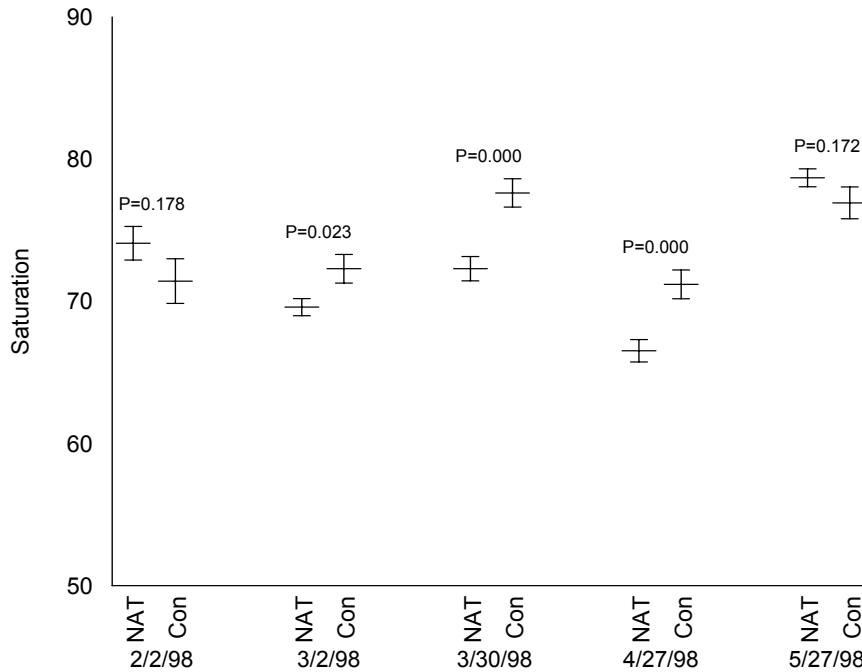


Figure 14. Mean saturation values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

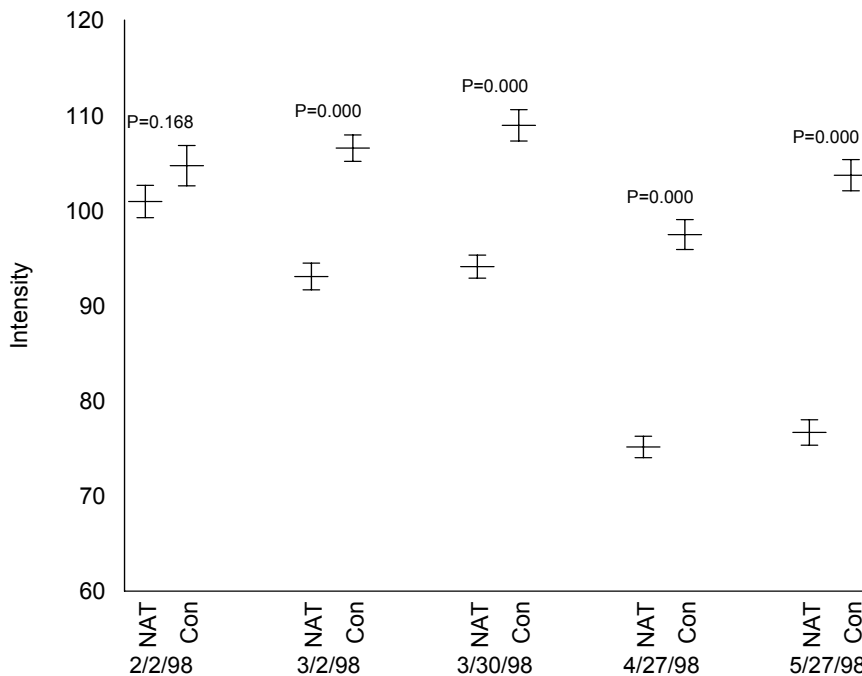


Figure 15. Mean intensity values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

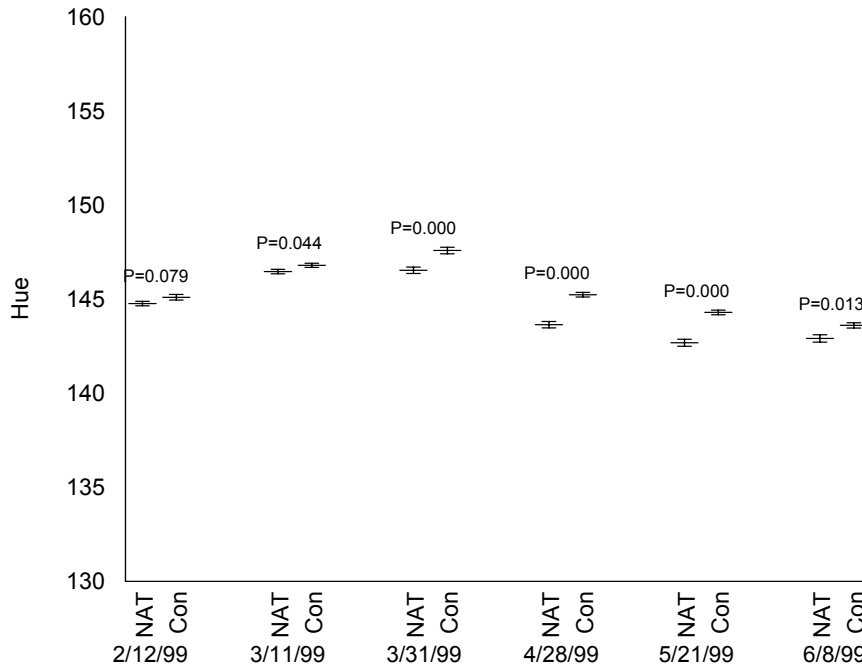


Figure 16. Mean hue values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, $n = 60$) or conventional (con, $n = 60$) raceways at Forks Creek Hatchery in 1999. P values are based on t -tests.

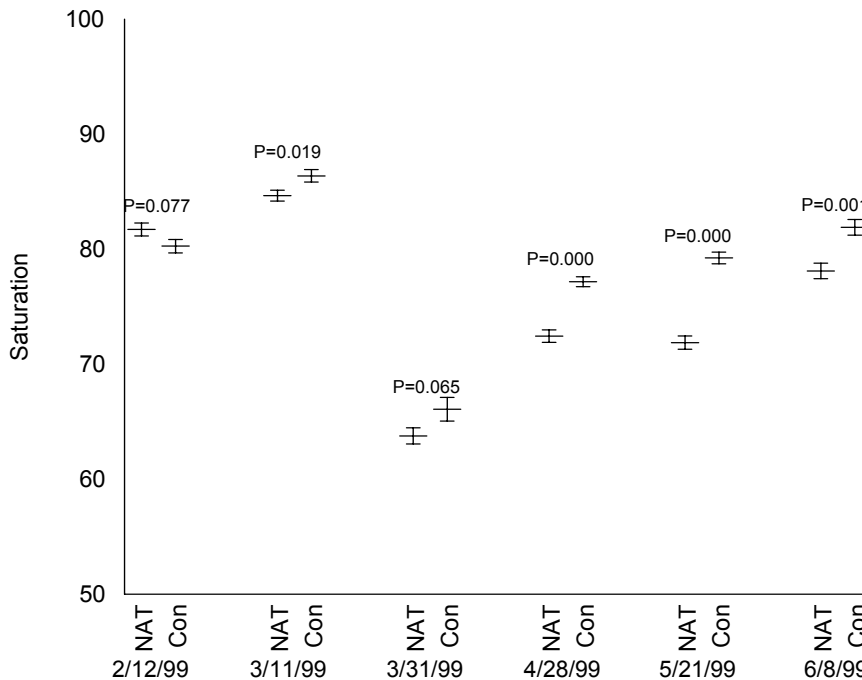


Figure 17. Mean saturation values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, $n = 60$) or conventional (con, $n = 60$) raceways at Forks Creek Hatchery in 1999. P values are based on t -tests.

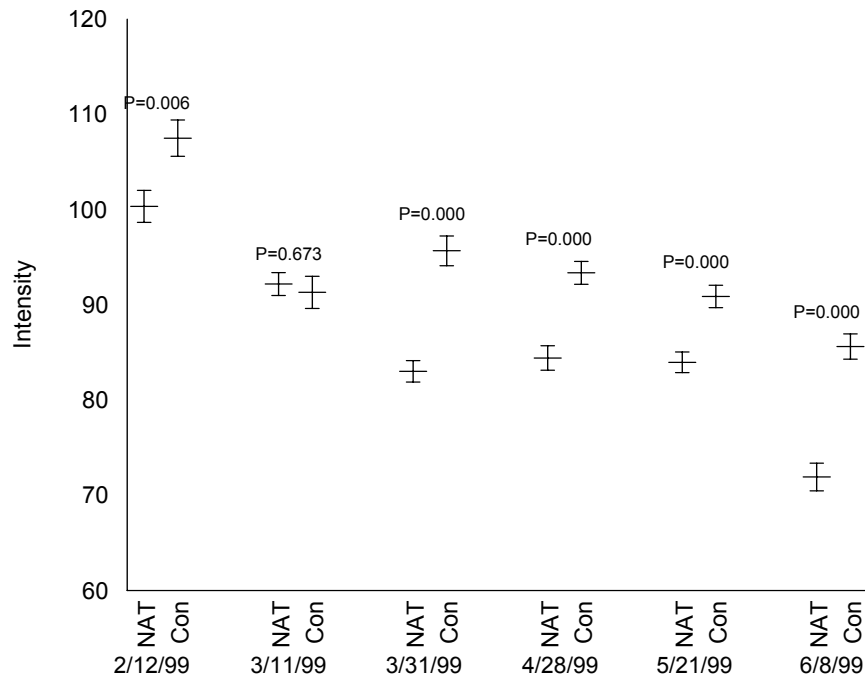


Figure 18. Mean intensity values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1999. P values are based on *t*-tests.

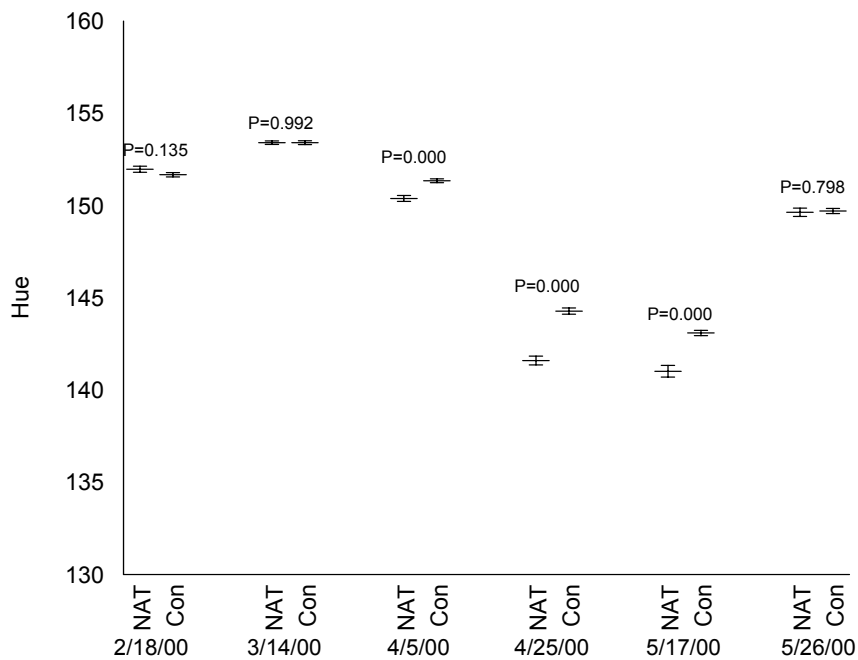


Figure 19. Mean hue values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 2000. P values are based on *t*-tests.

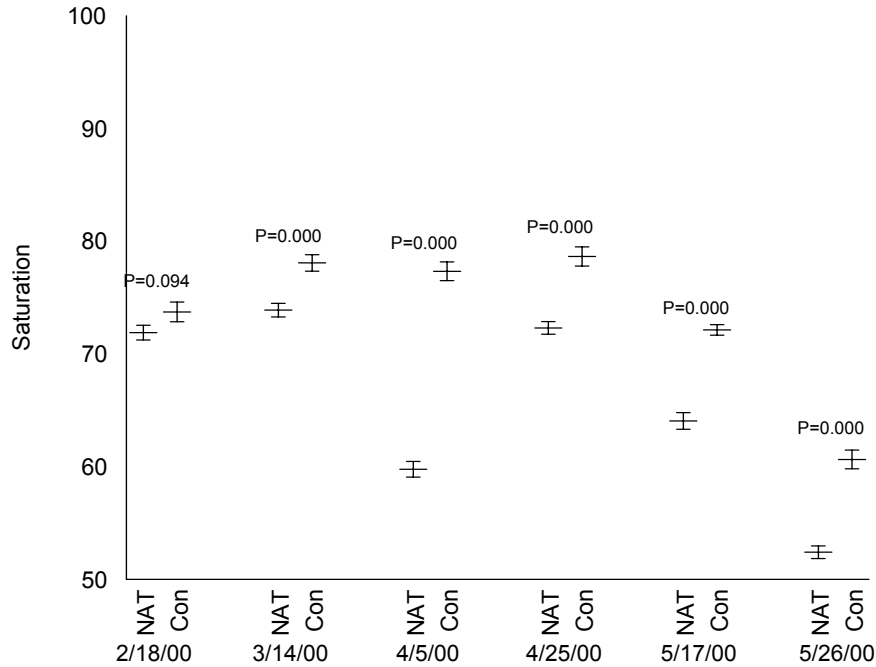


Figure 20. Mean saturation values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 2000. P values are based on *t*-tests.

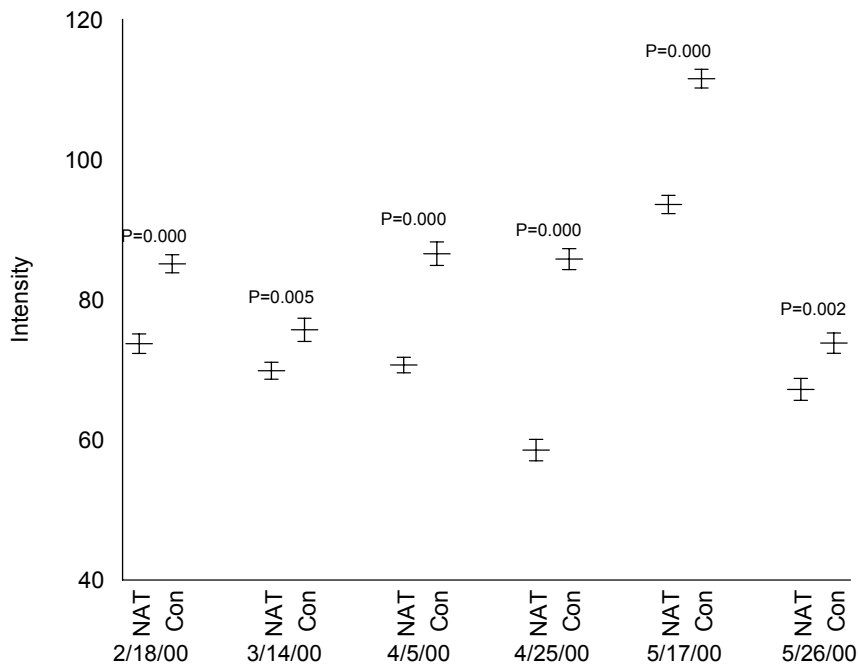


Figure 21. Mean intensity values (with standard error bars) of fall chinook salmon throughout rearing in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 2000. P values are based on *t*-tests.

In-culture Depth Distribution

In 1998, there were no statistically significant differences in the depth distribution of the fish in conventional and seminatural ponds ($P = 0.966$; Fig. 22). However, there was a significant ($P < 0.001$) interaction effect between rearing treatment and depth. Visually, a greater percentage of the conventionally-reared than seminaturally-reared chinook salmon were observed deeper in the water column in 1998.

In 1999, there was no interaction ($P = 0.993$) or rearing treatment ($P = 0.420$) effect on fish depth distribution (Fig. 23). However, there was a significant depth effect ($P < 0.001$) with the fish in both types of tanks primarily being observed higher in the water column. This may be an artifact of the video analyst being better able to distinguish between fish images that were higher in the water column than images that were lower in the water column.

In 2000, there was again no rearing treatment effect ($P = 0.965$) on fish depth distribution in the raceways (Fig. 24). There was again a significant difference ($P < 0.001$) in the percent of fish counted at the four different depths with the fewest fish being counted in the bottom grid. In this data set there was a significant interaction difference ($P = 0.037$) between rearing treatment and depth distribution.

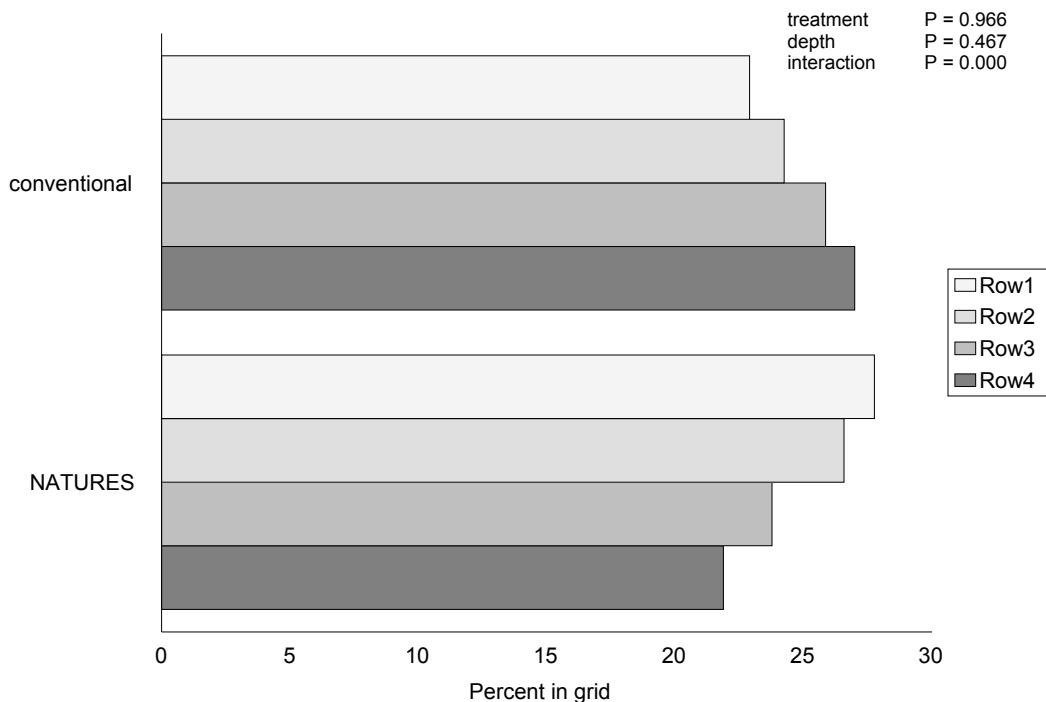


Figure 22. Depth distribution of fall chinook salmon reared in seminatural or conventional raceways at Forks Creek Hatchery in 1998. P values are based on two-way ANOVA.

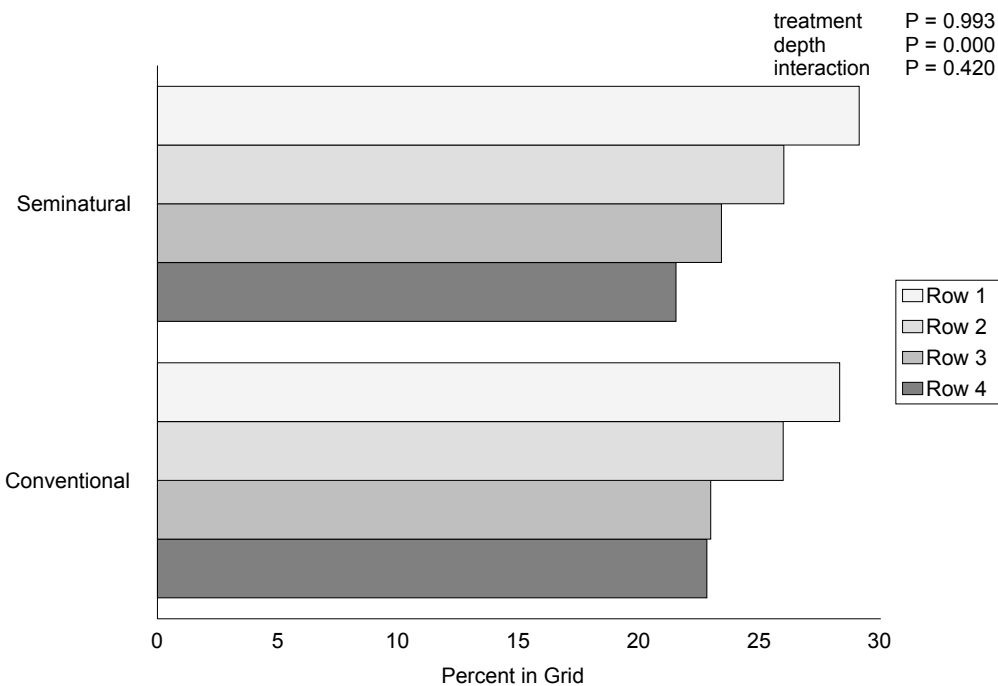


Figure 23. Depth distribution of fall chinook salmon reared in seminatural or conventional raceways at Forks Creek Hatchery in 1999. P values are based on two-way ANOVA.

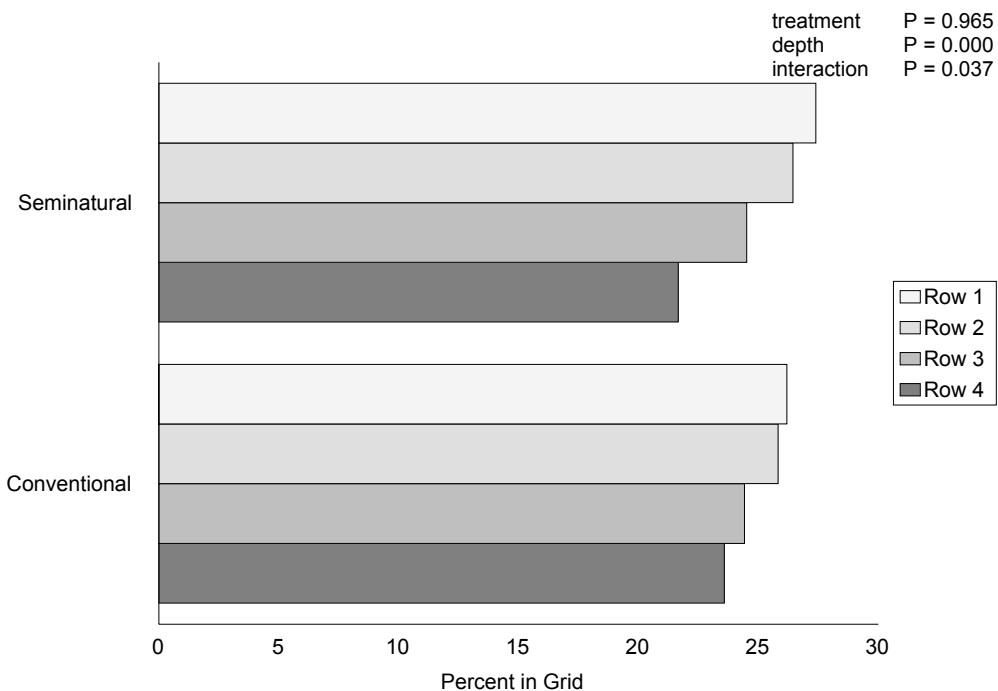


Figure 24. Depth distribution of fall chinook salmon reared in seminatural or conventional raceways at Forks Creek Hatchery in 2000. P values are based on two-way ANOVA.

Water Quality

Water quality sampling in production hatcheries was complicated by hatchery operations. Sampling and tagging that occurred during the first two water quality sampling dates might have influenced dissolved oxygen, pH, suspended solids, and total gas pressure.

The detailed results are presented in a series of tables by sampling date and parameter (Tables 1 through 5). Light levels data are presented separately in Figure 25.

Dissolved Oxygen (DO)

The minimum dissolved oxygen concentration was 7.3 mg/L. This occurred during the 12 April sampling when the fish were being CWT tagged. In general, the effluent dissolved oxygen was lower in the seminatural tanks (4 out of 5 sampling dates). The dissolved oxygen was significantly lower in the seminatural tanks 3 out of the 5 sampling dates. A significant portion of the oxygen consumption in the rearing tank was not due to the fish (see Table 2).

Total Gas Pressure (ΔP)

The effluent ΔP s appeared to be comparable. The influent ΔP s did vary over the day. The maximum observed value was 42 mm Hg.

Temperature (T)

Between 5 April and 24 May 2000, the influent temperature ranged from 7.9 to 12.6°C. During the first three sampling dates, the effluent temperature in both the seminatural and conventional tanks actually decreased and were not significantly different. In the fourth sampling date, both effluent temperatures increased but were not significantly different. In the last sampling date, the effluent temperatures were significantly different. The seminatural effluent temperature decreased by -0.21°C while the control increased by 0.02°C.

Total Ammonia Nitrogen (TAN)

The overall concentration of total ammonia nitrogen was low. The maximum observed value was 0.25 mg/L and was commonly less than 0.10 mg/L. During the last sampling date (24 May), the total ammonia nitrogen concentrations were significantly higher in the seminatural tank during both the morning and afternoon periods.

Un-ionized Ammonia Nitrogen (UIAN)

The un-ionized ammonia concentration were low (maximum = 0.30 $\mu\text{g/L}$). During the last sampling date (24 May), the un-ionized ammonia nitrogen concentrations were significantly higher in the seminatural tank during both the morning and afternoon periods.

pH

The influent pH ranged from 7.31-7.86 over the sampling period. The effluent pH were lower due to metabolic activities in the rearing tanks (6.90-7.48). During the last sampling date (24 May), the pH levels were significantly lower in the seminatural tank during both the morning and afternoon periods.

Total Suspended Solids (TSS)

The total suspended solids in the seminatural and conventional tank were less than 1 mg/L and appeared to be comparable. Because of these low levels, sample volumes of 1 liter or more were needed. The need for these large volumes limited the number of total suspended samples.

Light Levels

The light levels in the conventional tanks were reduced to 63% of the ambient levels by the bird netting (see Fig. 25). The light levels in the seminatural tanks were reduced to 33 to 43% compared to the light levels in the conventional tank (or 21 to 27% compared to the uncovered conventional tank). The light levels directly under a suspended tree were reduced by an additional factor of 2.

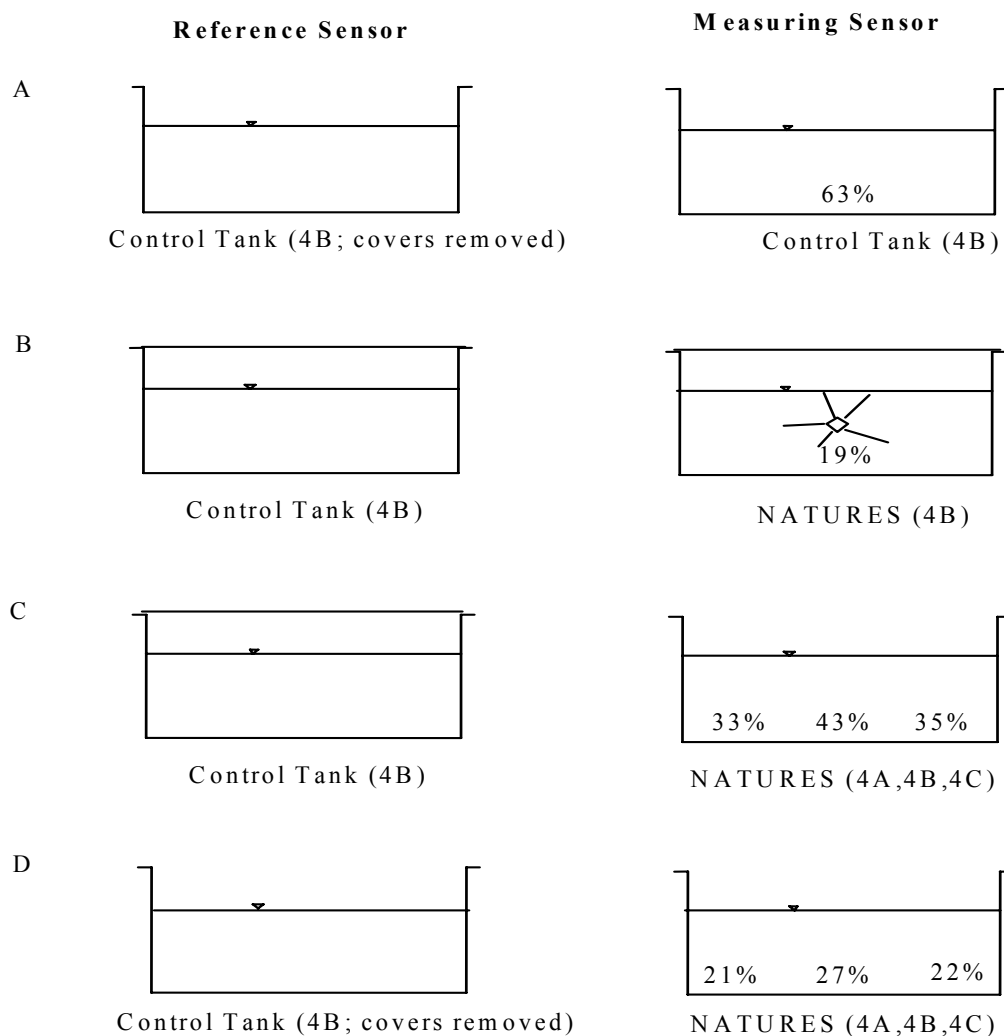


Figure 25. Ambient PAR (30-second averages) as a Percent of Reference Level (for location key, see Figure 1). A) Uncovered conventional vs. conventional; B) Conventional vs. seminatural under tree; C) Conventional vs. seminatural with tree removed; and D) Uncovered conventional vs. seminatural with tree removed.

Table 1. Results of water quality sampling at Forks Creek Hatchery on 5 April 2000. Fish were being sampled for photosampling. Data collection started with tank 21 and moved toward tank 26; tank 22 may have been more affected than tank 21. Pump for CWT trailer was located in tank 22, operated for about one hour. Fish were fed in middle of sampling.

	n	control mean \pm SE	n	seminatural mean \pm SE	P
Dissolved Oxygen – DO (mg/L)					
influent	5	11.520 \pm 0.112	5	12.170 \pm 0.281	0.064
effluent	5	10.416 \pm 0.051	5	9.896 \pm 0.250	0.076
Δ DO	5	-1.104 \pm 0.091	5	-2.274 \pm 0.184	0.000
Temperature – T (°C)					
influent	5	8.300 \pm 0.127	5	8.162 \pm 0.157	0.513
effluent	5	8.244 \pm 0.109	5	8.094 \pm 0.132	0.407
Δ T	5	-0.056 \pm 0.026	5	-0.068 \pm 0.031	0.775
Total Gas Pressure – Δ P (mm Hg)					
influent	5	10.000 \pm 1.000	5	10.800 \pm 1.281	0.636
effluent	5	10.200 \pm 2.059	5	11.200 \pm 1.497	0.705
$\Delta\Delta$ P	5	0.200 \pm 1.241	5	0.400 \pm 0.812	0.896
Total Ammonia Nitrogen – TAN (mg/L)					
influent	3	0.010 \pm 0.001	3	0.007 \pm 0.001	0.320
effluent	6	0.037 \pm 0.004	6	0.039 \pm 0.004	0.785
Un-ionized Ammonia – UIA (μ g/L)					
influent	3	0.067 \pm 0.003	3	0.037 \pm 0.007	0.016
effluent	6	0.162 \pm 0.022	5	0.150 \pm 0.025	0.734
PH					
influent	3	7.683 \pm 0.101	3	7.520 \pm 0.042	0.209
effluent	6	7.450 \pm 0.041	5	7.416 \pm 0.025	0.522
Total Suspended Solids – TSS (mg/L) *					
influent	2	n/a	4	n/a	n/a
effluent	6	n/a	6	n/a	n/a

* Based on 300 mL samples. Volumes not large enough for accurate analysis.

Table 2. Results of water quality sampling at Forks Creek Hatchery on 12 April 2000. Pump for CWT trailer was located in tank 22, operated during sampling. Fish were fed prior to sampling. Tank 24 was empty, and sampled for a seminatural baseline.

	n	control mean \pm SE	n	seminatural mean \pm SE	P
Dissolved Oxygen (DO)					
influent	5	11.060 \pm 0.073	5	11.110 \pm 0.255	0.855
effluent	5	8.330 \pm 0.251	5	7.880 \pm 0.174	0.179
Δ DO	5	-2.730 \pm 0.272	5	-3.230 \pm 0.269	0.227
Temperature (T)					
influent	5	11.540 \pm 0.246	5	11.390 \pm 0.281	0.699
effluent	5	11.380 \pm 0.244	5	11.240 \pm 0.294	0.724
Δ T	5	-0.160 \pm 0.040	5	-0.150 \pm 0.074	0.908
Total Gas Pressure CP – (mm Hg)					
influent	5	-2.200 \pm 1.562	5	-1.400 \pm 1.122	0.688
effluent	5	-4.800 \pm 1.655	5	-0.600 \pm 2.768	0.229
$\Delta\Delta$ P	5	-2.600 \pm 0.678	5	0.800 \pm 2.200	0.178
Total Ammonia Nitrogen – TAN (mg/L)					
influent	4	0.009 \pm 0.002	4	0.007 \pm 0.000	0.549
effluent	4	0.135 \pm 0.018	4	0.075 \pm 0.036	0.184
Un-ionized Ammonia – UIA (μ g/L)					
influent	4	0.025 \pm 0.009	4	0.018 \pm 0.003	0.437
effluent	4	0.210 \pm 0.027	4	0.120 \pm 0.056	0.196
PH					
influent	4	7.535 \pm 0.047	4	7.515 \pm 0.029	0.730
effluent	4	7.300 \pm 0.029	4	7.345 \pm 0.027	0.300
Total Suspended Solids – TSS (mg/L) *					
influent	1	1.700	1	1.300	n/a
effluent	4	2.100 \pm 0.424	2	1.850 \pm 0.250	0.722

* Based on 1,000-1,200 mL samples.

Table 3. Results of water quality sampling at Forks Creek Hatchery on 26 April 2000.

	n	control mean \pm SE	n	seminatural mean \pm SE	P
Dissolved Oxygen (DO)					
influent	5	11.970 \pm 0.118	5	11.910 \pm 0.056	0.658
effluent	5	10.150 \pm 0.153	5	10.482 \pm 0.165	0.179
Δ DO	5	-1.820 \pm 0.248	5	-1.428 \pm 0.213	0.265
Temperature (T)					
influent	5	9.366 \pm 0.162	5	9.238 \pm 0.175	0.606
effluent	5	9.292 \pm 0.153	5	9.146 \pm 0.182	0.556
Δ T	5	-0.074 \pm 0.029	5	-0.092 \pm 0.034	0.696
Total Gas Pressure Δ P – (mm Hg)					
influent	5	14.600 \pm 3.280	5	20.200 \pm 6.094	0.442
effluent	5	13.400 \pm 3.444	5	15.000 \pm 4.722	0.791
$\Delta\Delta$ P	5	-1.200 \pm 0.735	5	-5.200 \pm 1.625	0.055
Total Ammonia Nitrogen – TAN (mg/L)					
influent	3	0.006 \pm 0.001	2	0.008 \pm 0.001	0.463
effluent	3	0.094 \pm 0.005	3	0.098 \pm 0.002	0.594
Un-ionized Ammonia – UIA (μ g/L)					
influent	3	0.027 \pm 0.009	2	0.035 \pm 0.005	0.537
effluent	6	0.272 \pm 0.013	5	0.266 \pm 0.004	0.711
PH					
influent	3	7.447 \pm 0.007	3	7.453 \pm 0.007	0.519
effluent	6	7.272 \pm 0.017	5	7.250 \pm 0.012	0.343
Total Suspended Solids – TSS (mg/L) *					
influent			2	1.100 \pm 0.200	n/a
effluent	5	1.400 \pm 0.055	3	1.367 \pm 0.291	0.887

* Based on 1,200-1,400 mL samples.

Table 4. Results of water quality sampling at Forks Creek Hatchery on 11 May 2000.

	n	control mean \pm SE	n	seminatural mean \pm SE	P
Dissolved Oxygen (DO)					
influent	5	12.282 \pm 0.023	5	12.328 \pm 0.019	0.159
effluent	5	10.130 \pm 0.065	5	9.824 \pm 0.015	0.002
Δ DO	5	-2.152 \pm 0.080	5	-2.504 \pm 0.013	0.002
Temperature (T)					
influent	5	8.772 \pm 0.039	5	8.744 \pm 0.033	0.599
effluent	5	8.830 \pm 0.034	5	8.744 \pm 0.041	0.320
Δ T	5	0.058 \pm 0.015	5	0.030 \pm 0.016	0.230
Total Gas Pressure Δ P – (mm Hg)		no measurements this sample date			
Total Ammonia Nitrogen – TAN (mg/L)					
influent	6	0.014 \pm 0.002	6	0.013 \pm 0.001	0.577
effluent	6	0.225 \pm 0.010	6	0.236 \pm 0.008	0.423
Un-ionized Ammonia – UIA (μ g/L)					
influent	6	0.112 \pm 0.021	6	0.107 \pm 0.015	0.847
effluent	6	0.405 \pm 0.026	6	0.378 \pm 0.020	0.429
pH					
influent	6	7.460 \pm 0.063	6	7.460 \pm 0.055	0.907
effluent	6	7.103 \pm 0.033	6	7.052 \pm 0.020	0.209
Total Suspended Solids – TSS (mg/L)		no measurements this sample date			

Table 5. Results of water quality sampling at Forks Creek Hatchery on 24 May 2000.

	n	control mean \pm SE	n	seminatural mean \pm SE	P
Dissolved Oxygen (DO)					
influent	5	11.134 \pm 0.039	5	11.188 \pm 0.062	0.481
effluent	5	8.644 \pm 0.170	5	7.886 \pm 0.145	0.010
Δ DO	5	-2.490 \pm 0.147	5	-3.302 \pm 0.105	0.002
Temperature (T)					
influent	5	12.406 \pm 0.086	5	12.358 \pm 0.089	0.708
effluent	5	12.430 \pm 0.077	5	12.174 \pm 0.074	0.043
Δ T	5	0.024 \pm 0.015	5	-0.184 \pm 0.026	0.000
Total Gas Pressure Δ P – (mm Hg)		no measurements this sample date			
Total Ammonia Nitrogen – TAN (mg/L)					
influent	6	0.010 \pm 0.001	6	0.023 \pm 0.015	0.393
effluent	12	0.061 \pm 0.004	12	0.093 \pm 0.008	0.001
Un-ionized Ammonia – UIA (μ g/L)					
influent	6	0.068 \pm 0.011	6	0.057 \pm 0.015	0.541
effluent	12	0.136 \pm 0.018	11	0.195 \pm 0.010	0.011
pH					
influent	6	7.507 \pm 0.008	6	7.500 \pm 0.004	0.467
effluent	12	7.098 \pm 0.022	11	7.002 \pm 0.026	0.010
Total Suspended Solids – TSS (mg/L)		no measurements this sample date			

Fish Health

The fish health data for the two rearing treatments were very similar in 1997. The kidney streaks from both rearing treatments produced no pathogen colonies on the TSA agar plates. Although *N. salmincola* cysts were observed in the kidney smears from both treatments, the average cyst counts were similar and not significantly different ($P = 0.422$; Fig. 26). Enteric redmouth (*Yersinia ruckeri* infection) broke out in one of the conventional tanks in 1997 and produced some mortality. The fish in all four tanks were fed medicated (Romet) feed as soon as the outbreak was detected and diagnosed by WDFW fish health staff.

In 1998, the fish health sampling program was extended to include gross assessment of fin condition and internal organs. In these observations, the percentages of fish with fin or kidney problems were similar for both rearing treatments (Fig. 27). However, abnormal spleens were observed in more conventional than seminatural habitat fish, though not statistically significant ($P = 0.054$). As in 1997, none of the streaks on the TSA agar plates resulted in the growth of identifiable pathogen colonies. Enteric redmouth disease again broke out in one of the conventional raceways and all six raceways were fed medicated (Romet) feed. This immediately prevented subsequent mortality problems developing in the diseased raceway. In 1998, the fish were checked for *I. necator* and given a formalin bath just prior to release.

In 1999, a more extensive evaluation of fish health was conducted following the general protocols described by the Goede Index. There were no statistically significant differences in fin quality, with most fish from both rearing treatments falling into class 0, indicating good fin condition (Fig. 28). Although there were no statistically significant differences in opercle, gill, or spleen condition, the few problems observed all occurred in the conventionally-reared salmon (Fig. 28). There were no statistically significant differences in the proportion of fish falling into the four fat categories (Fig. 28). The mean hematocrit value for seminaturally-reared salmon was lower, though not significantly ($P = 0.055$), than that for conventionally-reared salmon (Fig. 29). The mean leukocrit value was significantly lower ($P < 0.001$) for seminaturally-reared fish (Fig. 30). Enteric redmouth disease occurred and was treated with twelve days of medicated feed in the weeks before release. Immediately following the medicated feed treatment, a formalin bath was administered due to the discovery of *I. necator*.

In 2000, as in 1999, none of the streaks on the TSA agar plates resulted in the growth of identifiable pathogen colonies. There were again no statistically significant differences in fin quality (Fig. 31). The eye, gill, and opercle condition of the two treatments did not statistically differ, although again the few problems that did occur were observed in the control samples. The mesenteric fat condition of both treatments was similar. If anything the control fish had slightly more fat than the seminaturally-reared fish. In 2000, all sampled spleens in both treatments appeared normal. The hindgut classification of the two rearing treatments was nearly identical, with only one control fish showing any problems at all. The only variable to differ significantly between treatments was the bile ($P = 0.003$). The color of the bile did not differ between treatments, but the seminaturally-reared fish had overall smaller gall bladders than the

conventionally-reared fish (Fig. 31). All the sampled fish in 2000 had normal kidneys, and the sex ratio of the sampled fish did not significantly differ. The mean hematocrits and plasma protein values for the two rearing treatments were very similar (Figs. 32 and 33). Overall, fish in the two rearing treatments appeared to be in good health with the few problems that cropped up occurring in the controls. A prophylactic formalin bath was administered just prior to PIT tagging. After PIT tagging, there was a major pathogen outbreak of enteric redmouth disease in control raceway 21. All the study fish on station were successfully medicated with 2% medicated Romet feed at this time.

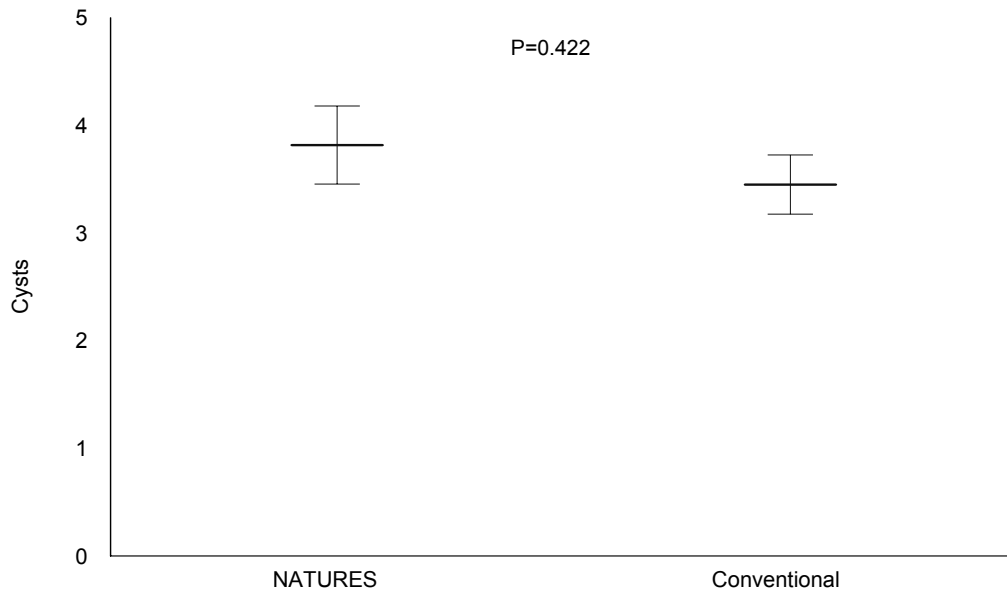


Figure 26. Mean number of *Nanophyetus salmincola* cysts found in kidney smears of fall chinook salmon reared in seminatural (n = 60) or conventional (n = 60) raceways at Forks Creek Hatchery in 1997. P values are based on *t*-tests.

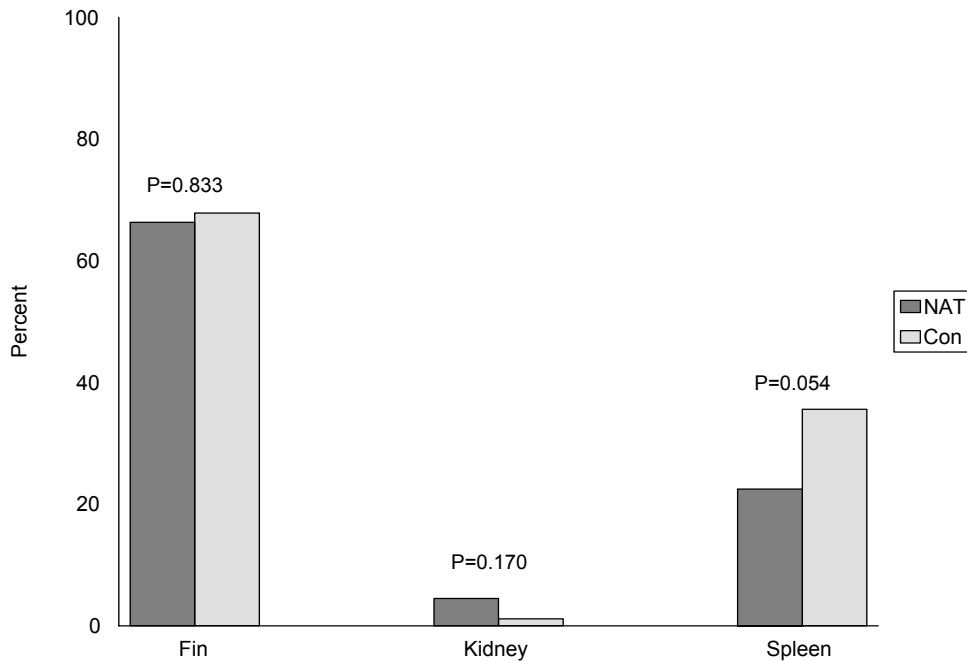


Figure 27. Percentage of fall chinook salmon with abnormal health conditions. Fish were reared in seminatural (NAT, n = 60) or conventional (con, n = 60) raceways at Forks Creek Hatchery in 1998. P values are based on *t*-tests.

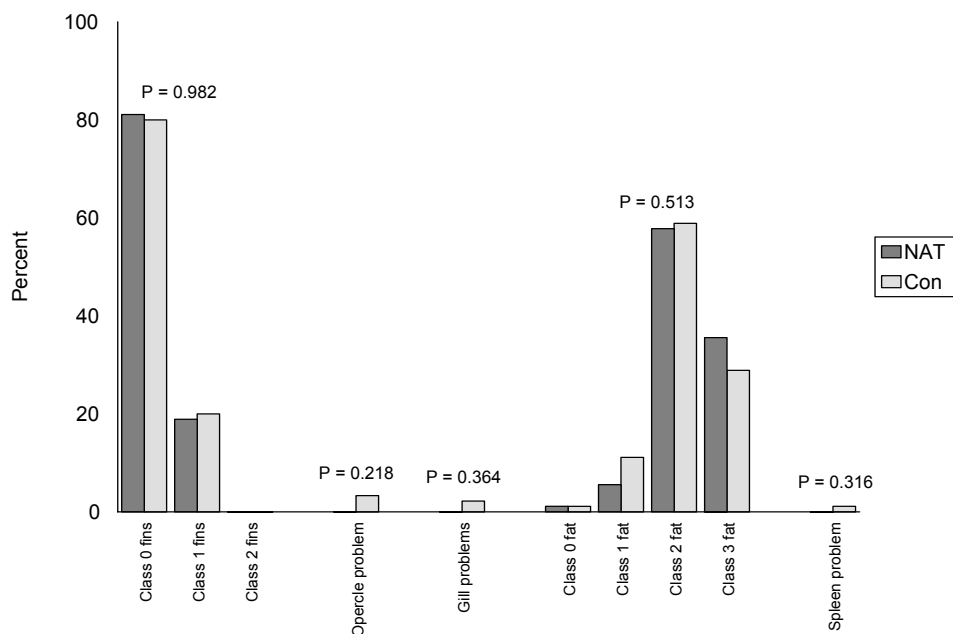


Figure 28. Percentage of fish falling into each Goede health index category. Fish were reared in seminatural (NAT, n = 90) or conventional (con, n = 90) raceways at Forks Creek Hatchery in 1999. P values are based on chi-square analysis.

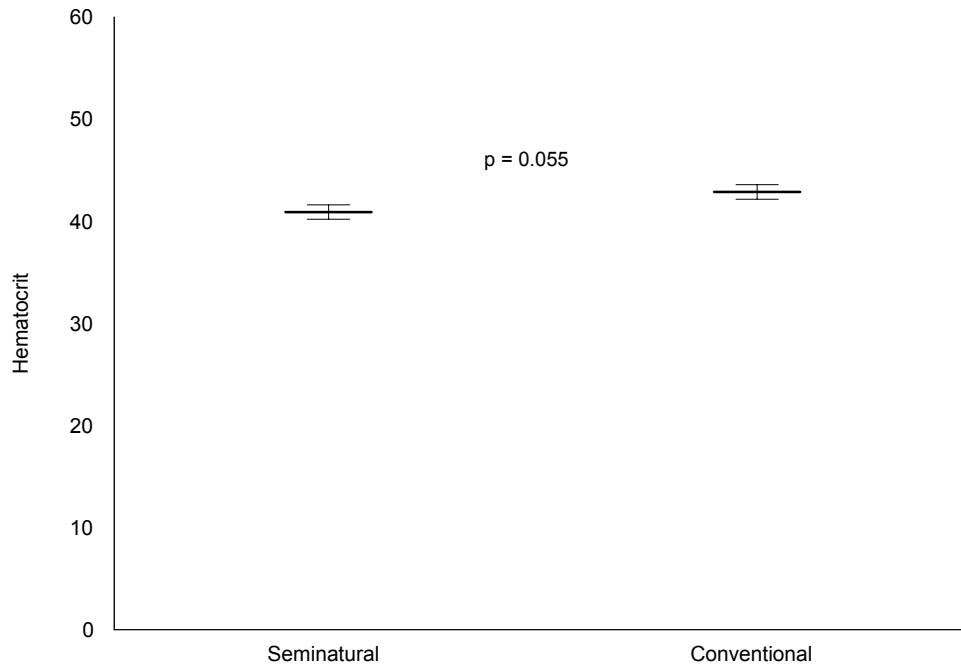


Figure 29. Mean hematocrit values of fall chinook salmon reared in seminatural (n = 90) or conventional (n = 90) raceways at Forks Creek Hatchery in 1999. P value is based on a *t*-test.

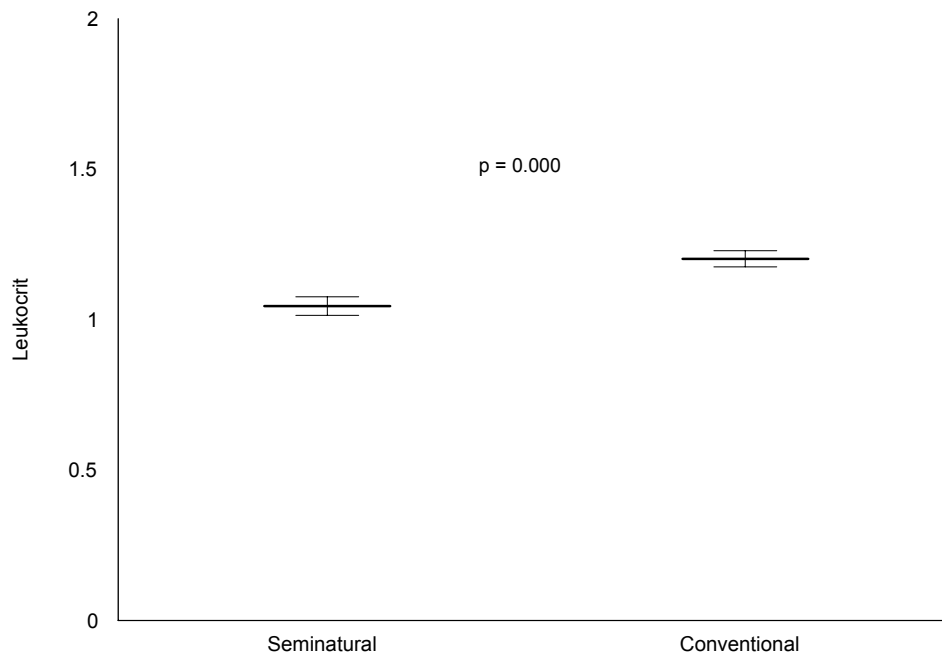


Figure 30. Mean leukocrit values of fall chinook salmon reared in seminatural (n = 90) or conventional (n = 90) raceways at Forks Creek Hatchery in 1999. P value is based on a *t*-test.

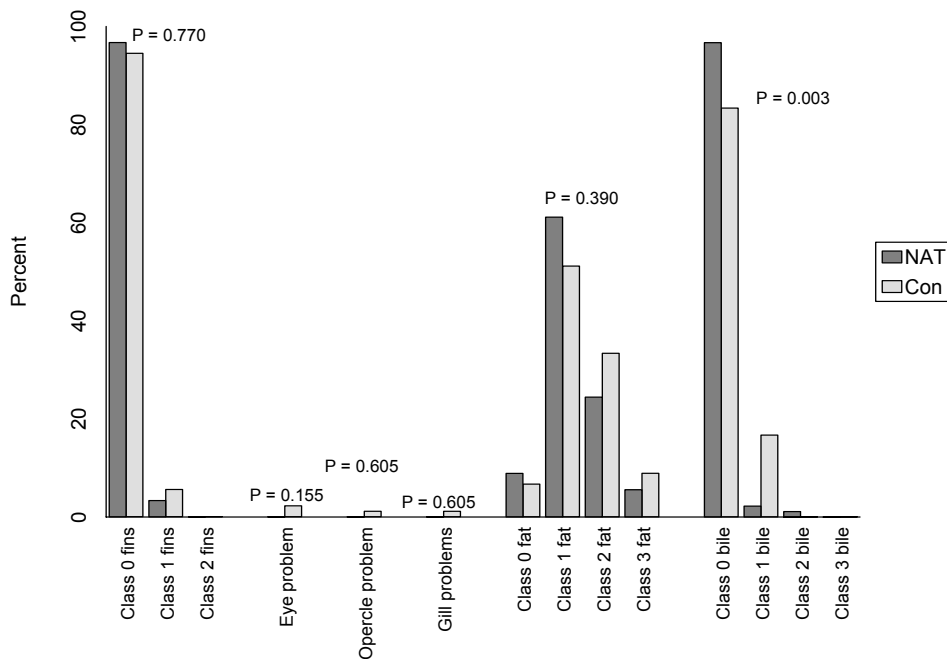


Figure 31. Percentage of fish falling into each Goede health index category. Fish were reared in seminatural (NAT, n = 90) or conventional (con, n = 90) raceways at Forks Creek Hatchery in 2000. P values are based on chi-square analysis.

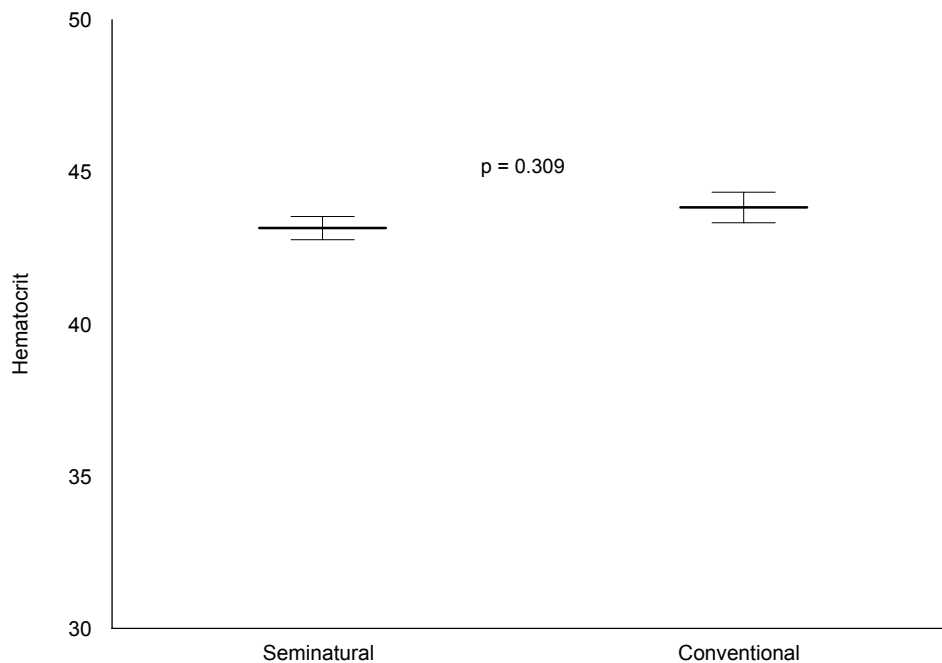


Figure 32. Mean hematocrit values of fall chinook salmon reared in seminatural (n = 90) or conventional (n = 90) raceways at Forks Creek Hatchery in 2000. P value is based on a *t*-test.

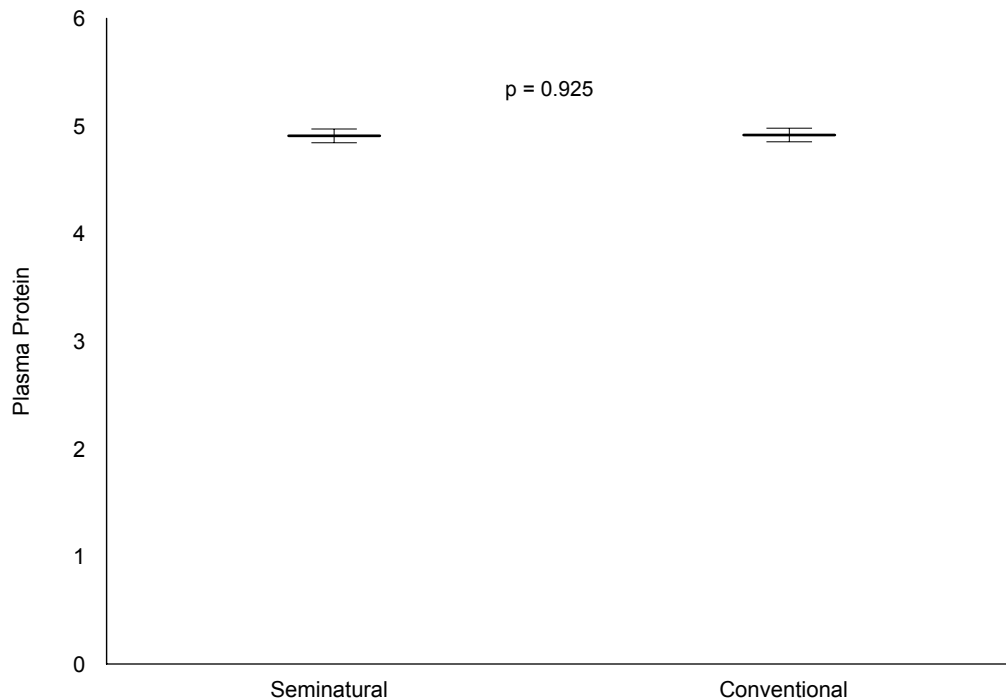


Figure 33. Mean plasma protein values of fall chinook salmon reared in seminatural (n = 90) or conventional (n = 90) raceways at Forks Creek Hatchery in 2000. P value is based on a *t*-test.

Travel Time

On average, fish were recaptured at the weir in less than 2.9 days in 1997 (Fig. 34). Rearing treatment was not a significant factor affecting the time it took fish to reach the weir ($P = 0.199$), but release date was a significant factor that affected travel time ($P = 0.000$). Fish in the second release generally reached the weir more rapidly than fish in the first release. There was no significant interaction ($P = 0.904$) between treatment and release date in the 1997 experiment.

On average fish took longer (4.3 or more days) to reach the weir in 1998 than 1997 (Fig. 35). However, as in the previous year, there was no significant effect of rearing treatment on travel time ($P = 0.453$), but there was again a statistically significant effect of release date on travel time ($P = 0.000$). In general, fish in the first release took the longest time to reach the weir, fish in the second release took a slightly shorter time, and the fish in the third release reached the weir in the least time. Again there was no statistically significant interaction between rearing treatment and release date ($P = 0.132$).

In 1999, there was a significant effect of rearing treatment on travel time ($P = 0.008$), as well as of release date ($P = 0.000$; Fig. 36). Additionally, there was an interaction between release and treatment factors for travel time ($P = 0.046$). Mean travel times for both treatments in both of the first two releases averaged about 2.6 days, whereas the third release group had travel times of 3.9 and 3.3 days for control and seminatural habitat fish, respectively.

On average fish took approximately 5 days to reach the weir in 2000 (Fig. 37). In 2000, there was no significant effect of rearing treatment on travel time ($P = 0.130$), but there was a statistically significant effect of release date on travel time ($P = 0.002$). There was no statistically significant interaction between rearing treatment and release date ($P = 0.400$). None of the individual 2000 releases had a significant difference in travel time by treatment.

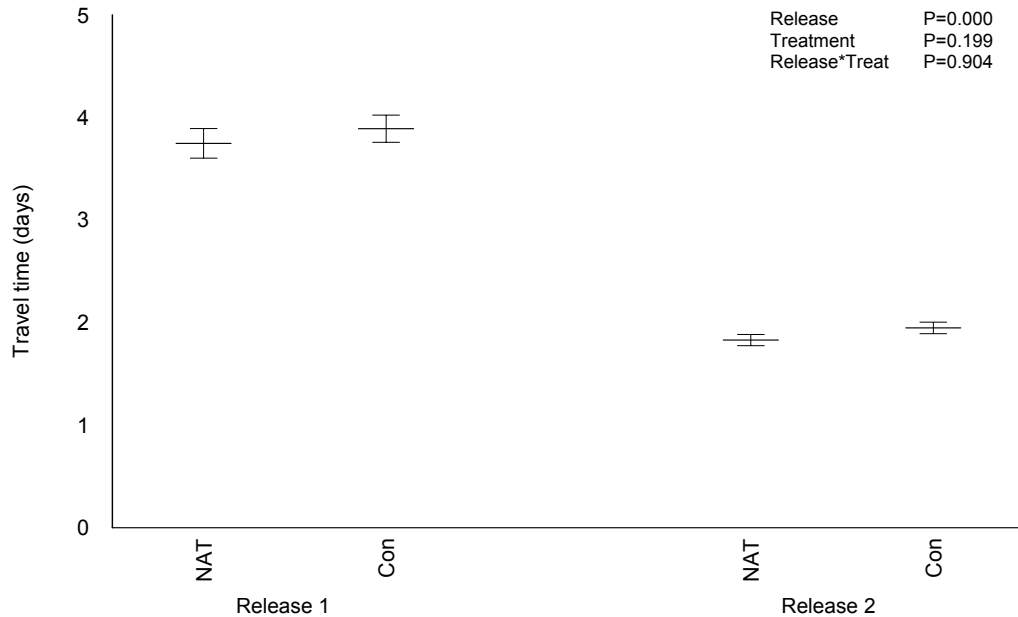


Figure 34. Mean travel time (with standard error bars) for outmigrating fall chinook salmon reared in seminatural (NAT, $n = 1042$) or conventional (con, $n = 1036$) raceways at Forks Creek Hatchery in 1997. Travel time is measured as days from release above hatchery to recapture at a weir downstream. P values are based on two-factor ANOVA.

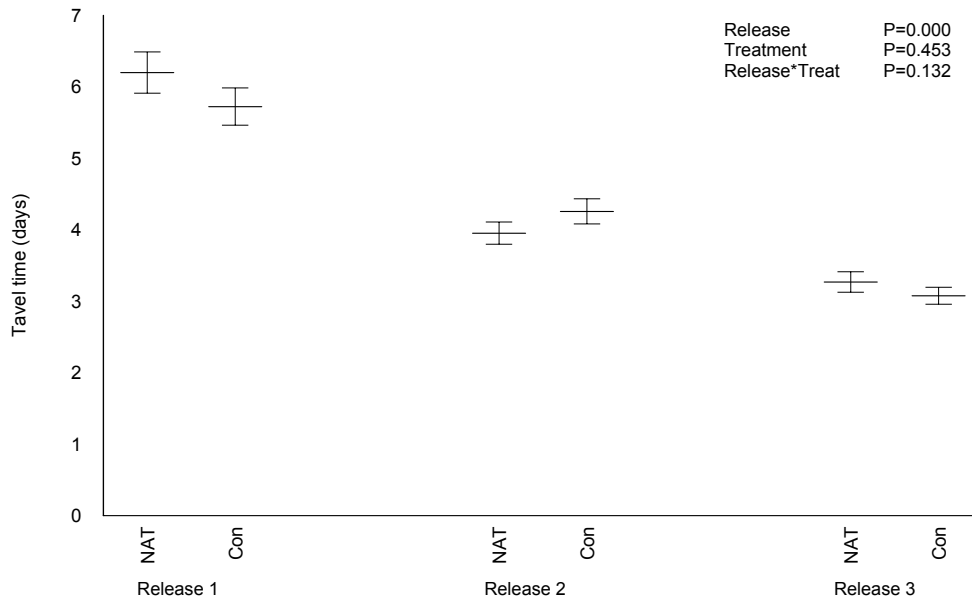


Figure 35. Mean travel time (with standard error bars) for outmigrating fall chinook salmon reared in seminatural (NAT, n = 988) or conventional (con, n = 890) raceways at Forks Creek Hatchery in 1998. Travel time is measured as days from release above hatchery to recapture at a weir downstream. P values are based on two-factor ANOVA.

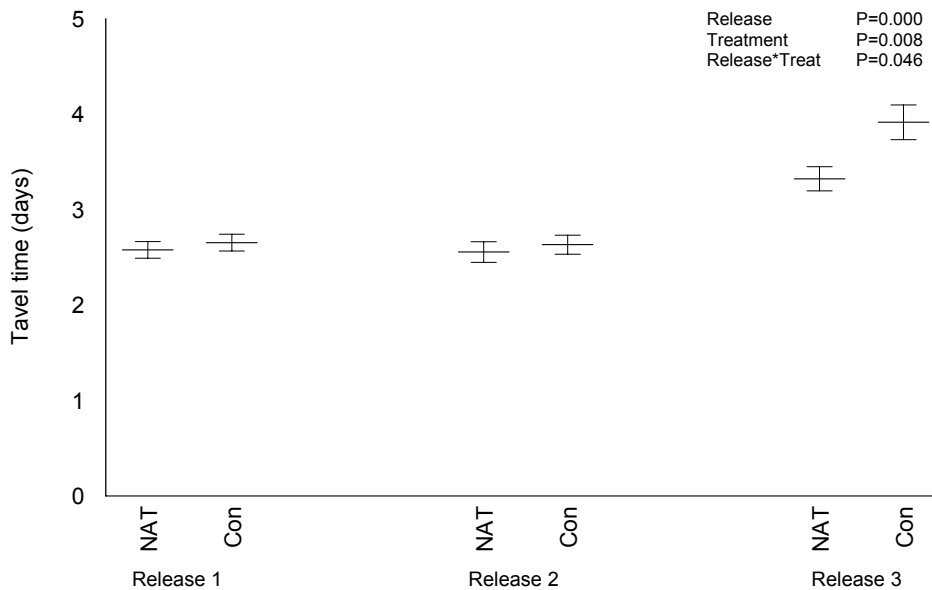


Figure 36. Mean travel time (with standard error bars) for outmigrating fall chinook salmon reared in seminatural (NAT, n = 1075) or conventional (con, n = 869) raceways at Forks Creek Hatchery in 1999. Travel time is measured as days from release above hatchery to recapture at a weir downstream. P values are based on two-factor ANOVA.

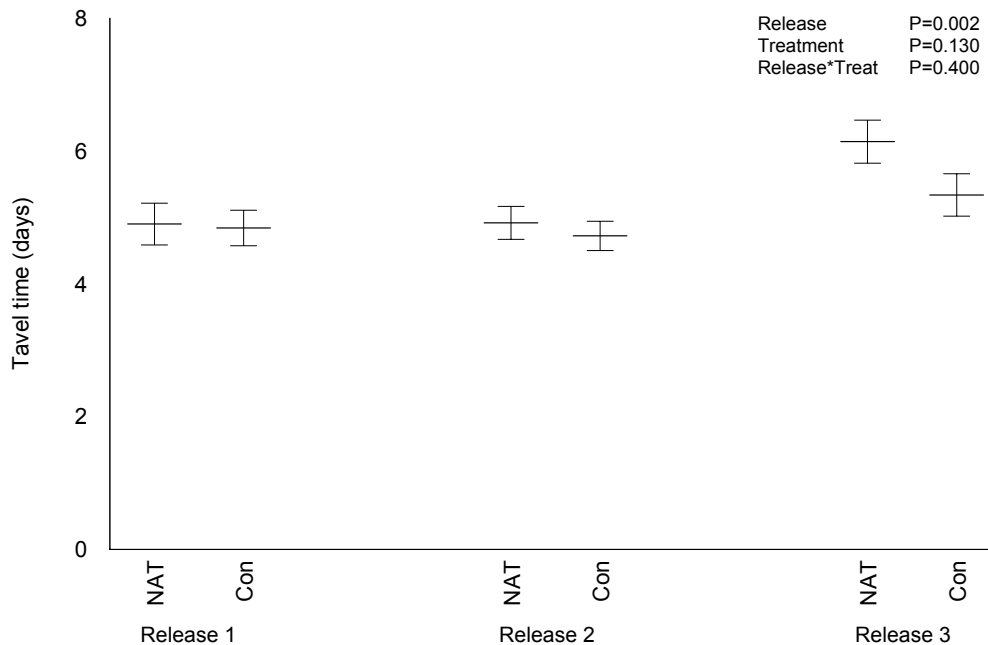


Figure 37. Mean travel time (with standard error bars) for outmigrating fall chinook salmon reared in seminatural (NAT, n = 1209) or conventional (con, n = 1119) raceways at Forks Creek Hatchery in 2000. Travel time is measured as days from release above hatchery to recapture at a weir downstream. P values are based on two-factor ANOVA.

Instream Survival

The majority of the fish released in 1997, 1998, 1999, and 2000 survived downstream migration to the weir at Forks Creek Hatchery. Overall instream survival was higher for 1997 and 2000 and lower for both 1998 and 1999.

In both 1997 releases, slightly more seminaturally-reared than conventionally-reared chinook salmon were recovered at the weir, but the difference was not statistically significant (Fig. 38). The combined results of the two 1997 releases had higher survival for seminaturally-reared fish, but also not at statistically significant levels ($P = 0.091$).

In two of the three 1998 releases, significantly more seminaturally-reared than conventionally-reared fish were recovered at the weir (Fig. 39). The other 1998 release mimicked this recovery pattern of more fish from seminatural than conventional habitat, but the difference was not statistically significant. The pooled 1998 instream survival data was also significantly in favor of seminaturally-reared fish ($P < 0.001$).

In 1999, significantly more seminaturally-reared than conventionally-reared chinook salmon were recovered in all three releases, as well as at the pooled level ($P < 0.001$; Fig. 40).

In 2000, slightly more seminaturally-reared than control fish were recovered at the weir (Fig. 41). However, as in 1997, the differences were miniscule and not statistically significant ($P = 0.604$ for all three releases combined).

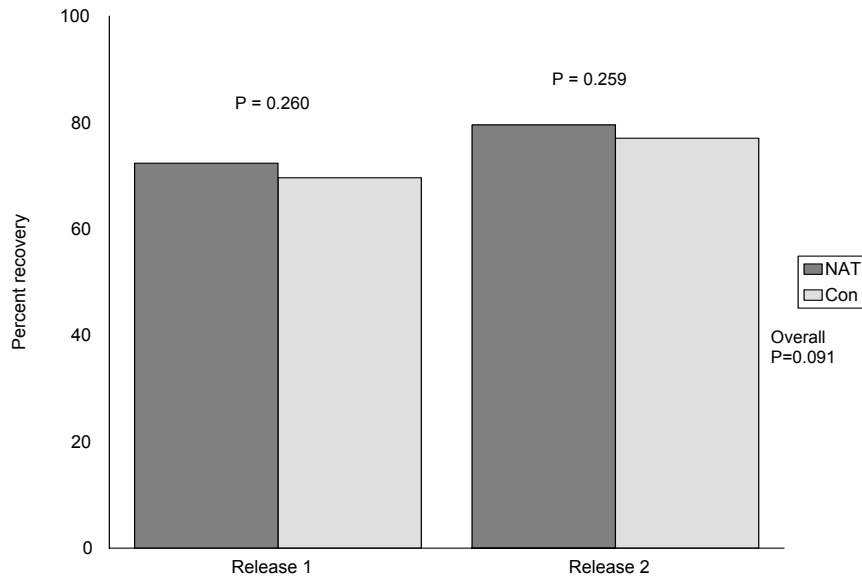


Figure 38. Percent of fall chinook salmon recovered in smolt-to-smolt survival evaluations. Fish were reared in seminatural (NAT, $n = 1042$) or conventional (con, $n = 1036$) raceways at Forks Creek Hatchery in 1997. P values are based on chi-square analysis.

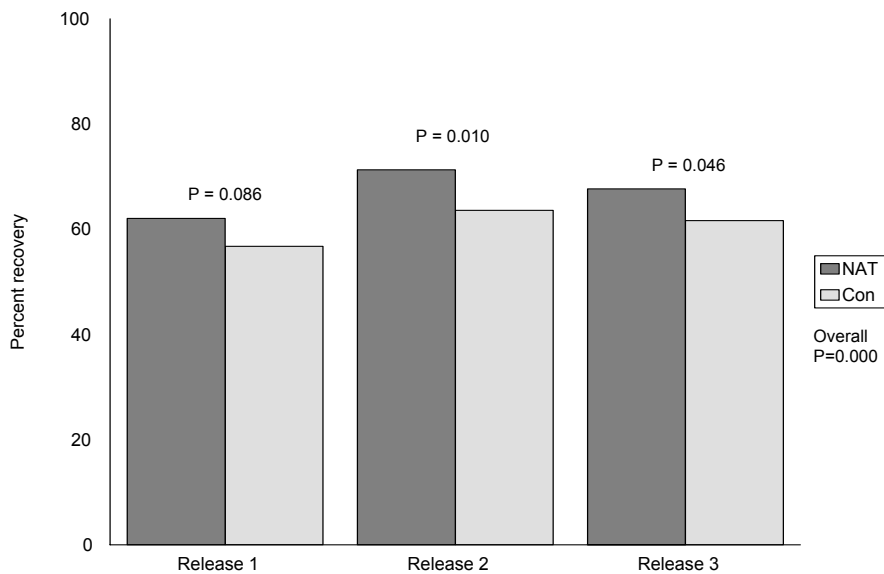


Figure 39. Percent of fall chinook salmon recovered in smolt-to-smolt survival evaluations. Fish were reared in seminatural (NAT, $n = 1001$) or conventional (con, $n = 899$) raceways at Forks Creek Hatchery in 1998. P values are based on chi-square analysis.

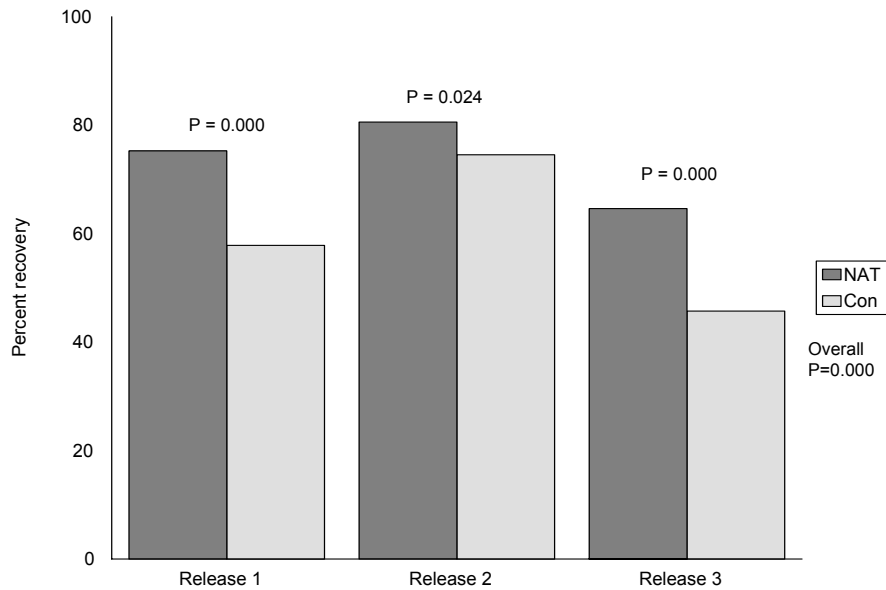


Figure 40. Percent of fall chinook salmon recovered in smolt-to-smolt survival evaluations. Fish were reared in seminatural (NAT, $n = 1464$) or conventional (con, $n = 1466$) raceways at Forks Creek Hatchery in 1999. P values are based on chi-square analysis.

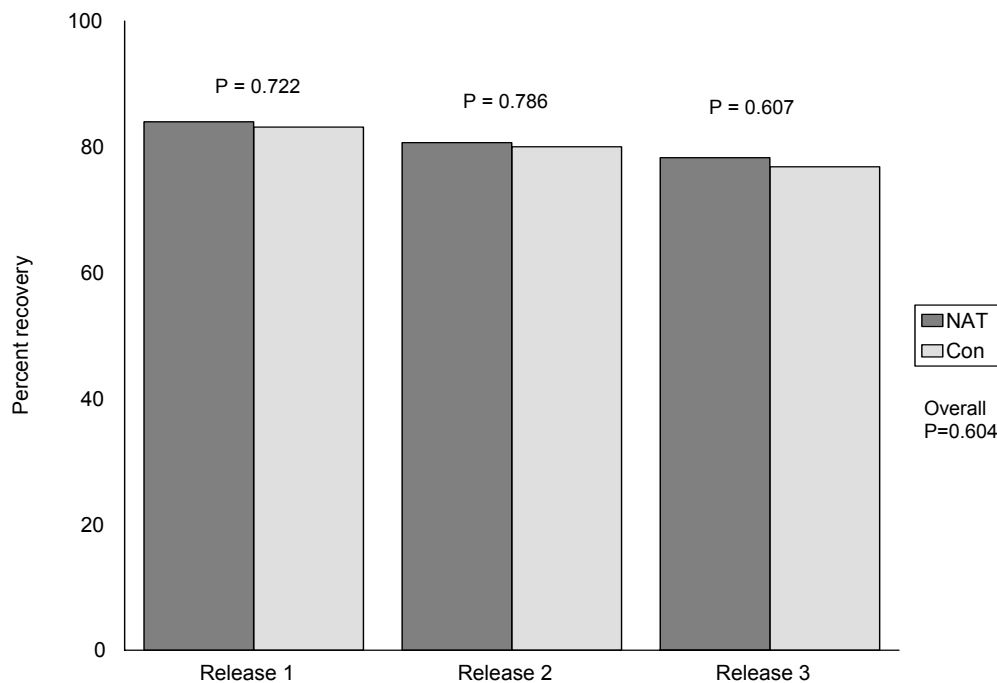


Figure 41. Percent of fall chinook salmon recovered in smolt-to-smolt survival evaluations. Fish were reared in seminatural (NAT, $n = 1493$) or conventional (con, $n = 1395$) raceways at Forks Creek Hatchery in 2000. P values are based on chi-square analysis.

Predator Avoidance Bioassays

A total of 29 fish recovered from the predator bioassay experiments in 2000 did not contain coded-wire tags. In nine cases, it was possible to determine to which treatment the missing tags belonged by elimination. For instance, if all ten tags from one treatment were recovered, then the missing tags necessarily belonged to the other treatment. Similar logic was used wherever possible to determine the treatment of both non-tagged and eaten fish. The eighteen fish whose treatment could not be determined by logic alone were estimated. This represented 1.67% of the fish recovered. Using the quality control sheets from WDFW, we found that conventional raceways averaged 2.23% tag-loss, and seminatural raceways averaged 3.57% tag-loss. Using these figures, seminaturally-reared fish represent 61.55% of non-tagged fish, with the other 38.45% being control fish. These percentages were used to approximate the remaining data. First, trials were separated according to arena. Second, the number of unknowns per condition within each arena was calculated (3 killed in the barren arena trials, for example). Third, the quality control numbers were applied to these unknowns to calculate our estimates (2 seminatural and 1 conventional, continuing our example). Once these estimates were calculated, the numbers of fish per treatment consumed by the predator were determined. This was done by subtracting the total numbers killed, scarred, and unscarred per treatment from the total number of fish used.

Data analysis was via 2×2 contingency tables. Initially, data was separated by arena, and then heterogeneity chi-square tests were used to determine if data from both arenas could be pooled. It was determined that pooling was allowed.

Data was analyzed in several different ways. Fish were originally categorized as either killed, scarred, unscarred, or eaten. For the sake of the analysis, fish could also be grouped as dead (killed + eaten) or alive (scarred + unscarred), and attacked (killed + eaten + scarred) or not attacked (unscarred).

Comparing predator avoidance (attacked versus not attacked; Fig. 42), no treatment difference was detected in either the barren arena ($P = 0.110$), or the stream arena ($P = 0.176$). However, when the data was pooled, a statistically significant treatment difference was detected in predator avoidance ability ($P = 0.037$). Predator evasion success was not significantly affected by arena (Fig. 43). Outcome by arena did not differ significantly for either seminaturally-reared ($P = 0.191$) or conventionally-reared ($P = 0.280$) fish. When pooled, data still showed no statistical significance in predator avoidance by arena ($P = 0.092$).

Comparing survival (dead versus alive; Fig. 44), fish in the barren arena did not differ by treatment ($P = 0.114$), nor did fish in the stream arena ($P = 0.639$). Pooling the data, there was still no treatment difference in survival ($P = 0.144$). Breaking data down and comparing dead versus scarred versus unscarred (Fig. 45), no difference was detected in either the barren ($P = 0.187$) or the stream ($P = 0.396$) arenas. Likewise, no significant difference was found in pooled data ($P = 0.099$).

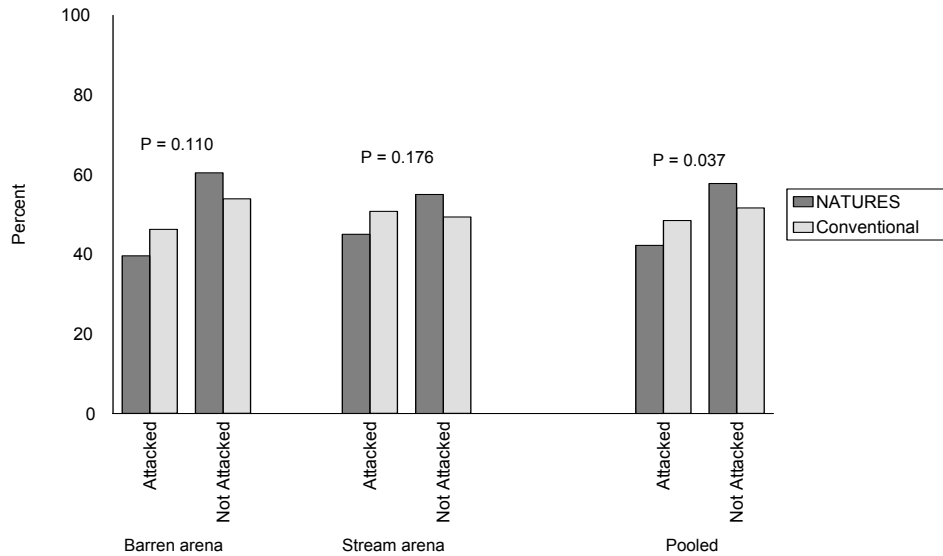


Figure 42. Predation avoidance, expressed as percentage and grouped by treatment, of Forks Creek fall chinook salmon in Manchester predation bioassays, 2000. Fish reared in seminatural and conventional raceways were tested in a barren arena (n = 288 per treatment) and a stream-like arena (n = 280 per treatment). P values are based on chi-square analysis.

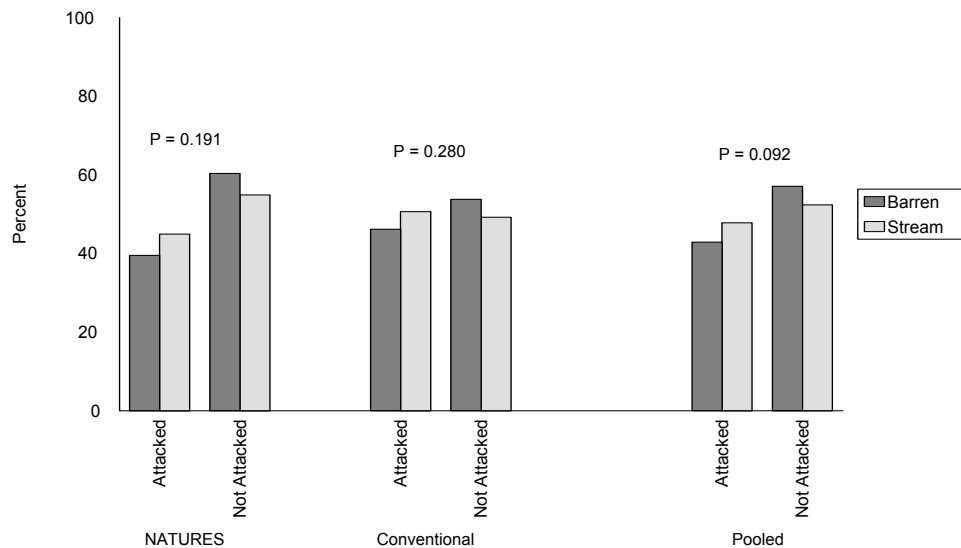


Figure 43. Predation avoidance, expressed as percentage and grouped by arena, of Forks Creek fall chinook salmon in Manchester predation bioassays, 2000. Fish reared in seminatural and conventional raceways were tested in a barren arena (n = 288 per treatment) and a stream-like arena (n = 280 per treatment). P values are based on chi-square analysis.

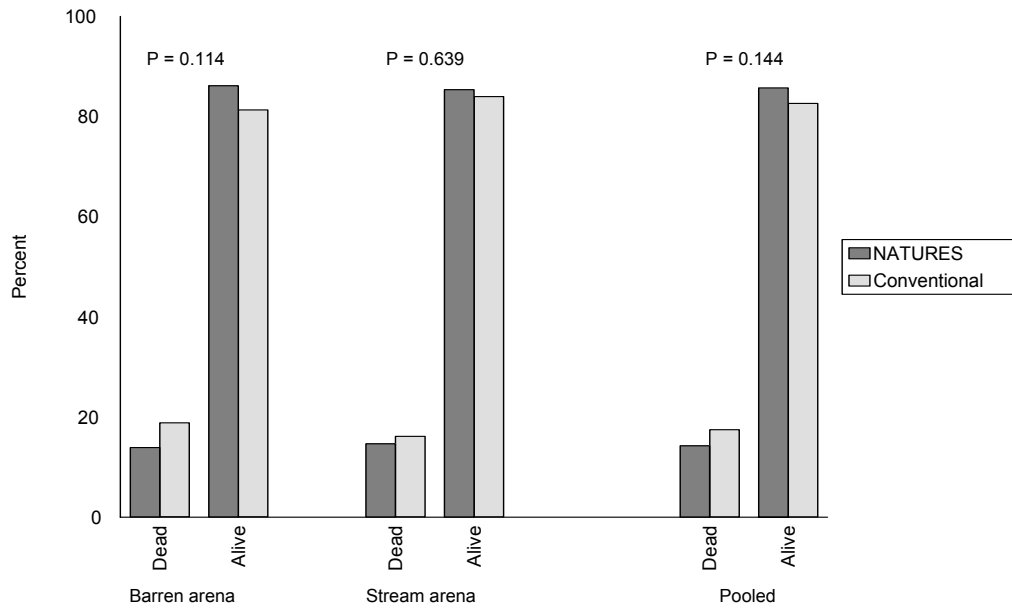


Figure 44. Percent survival of fall chinook salmon in Manchester predation bioassays, 2000. Fish reared in Forks Creek seminatural and conventional raceways were tested in a barren arena (n = 288 per treatment) and a stream-like arena (n = 280 per treatment). P values are based on chi-square analysis.

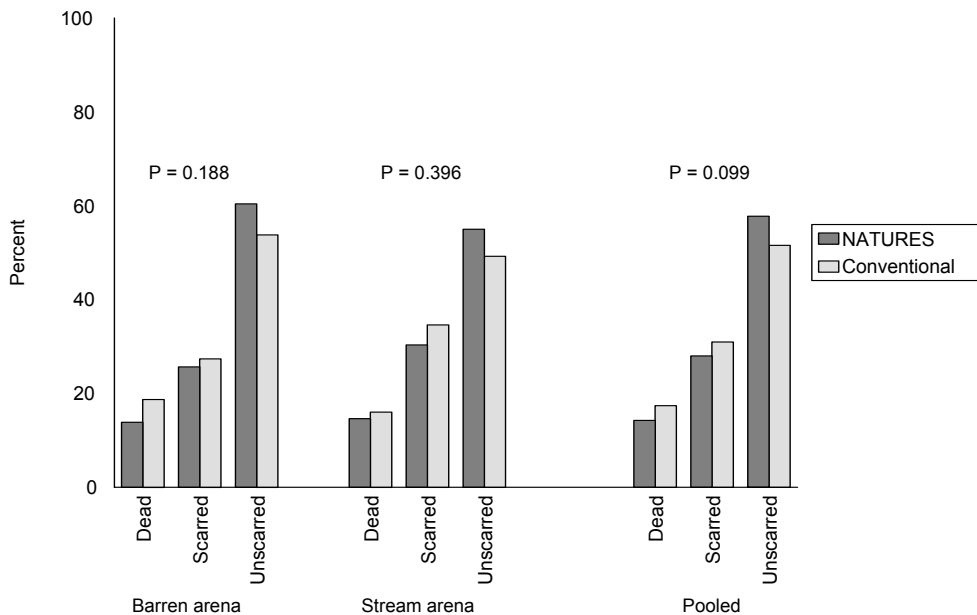


Figure 45. Categorical results, expressed as percentage and grouped by arena, of Forks Creek fall chinook salmon in Manchester predation bioassays, 2000. Fish reared in seminatural and conventional raceways were tested in a barren arena (n = 288 per treatment) and a stream-like arena (n = 280 per treatment). P values are based on chi-square analysis.

Smolt-to-Adult Survival

In 1997, more than 45,000 coded-wire tagged fish were released from each raceway (Table 6) for a total of more than 95,000 fish released per treatment. In 1998, more than 32,000 fish were released from each raceway, for a total of more than 99,000 fish being released per treatment. In 1999, more than 32,000 coded-wire tagged fish were released from each raceway for a total of more than 101,000 fish released per treatment. In 2000, more than 33,000 fish were released from each raceway, for a total of at least 102,000 fish being released per treatment.

Table 6. Coded-wire tagged releases of fall chinook salmon from Forks Creek Hatchery NATURES project in 1997, 1998, 1999, and 2000.

	Raceway 21	Raceway 22	Raceway 23	Raceway 24	Raceway 25	Raceway 26
Treatment	control	seminatural	control	seminatural	control	seminatural
1997						
CWT + Ad clip	44,172	46,258	47,589	45,368	--	--
CWT only	2,068	2,235	1,405	1,773	--	--
1998						
CWT + Ad clip	33,154	34,008	33,267	32,507	32,606	33,994
CWT only	194	0	67	66	97	34
1999						
CWT + Ad clip	32,488	34,899	35,030	34,935	33,789	36,234
CWT only	0	0	0	0	0	0
2000						
CWT + Ad clip	34,372	34,666	34,711	34,274	34,484	33,425
CWT only	0	0	0	71	0	105

Of the 95,234 conventionally-reared and coded-wire-tagged fish released in 1997, 13 were recovered in 1999 and 26 were recovered in 2000 (Table 7). Of the 95,634 seminaturally-reared fish in CWT releases in 1997, 8 were recovered in 1999 and 34 in 2000. This equates to an overall smolt-to-adult survival for control fish of 0.041%, and 0.044% for seminaturally-reared fish. Observed CWT recoveries were not significantly different ($P = 0.753$), according to 2×2 contingency table analysis. Fall chinook salmon may mature at age 6-7. Therefore, adult returns from 1997 releases cannot be assumed complete until 2003.

In 1998, 99,318 conventionally-reared and 100,609 seminaturally-reared fish were released with coded-wire tags. The 1999 recoveries consisted of a single conventionally-reared fish (Table 7). In 2000, 10 control and 10 seminatural habitat fish

were recovered. This is equivalent to a smolt-to-adult survival of 0.011% for conventionally-reared fish and 0.0099% for seminaturally-reared fish. Observed CWT recoveries were not significantly different ($P = 0.805$). Recoveries of fall chinook salmon released in 1998 may return as late as 2003, and, therefore, these data are preliminary.

Of those fish released in 1999, WDFW has records of 2 returning to the hatchery rack in 2000, one originating from control and one from seminatural habitat.

The return and recovery data available at this time is incomplete and therefore inconclusive. It will be 2003 before full analysis of the 1997 releases will be possible, 2004 for 1998 releases, and so on. In addition, the information included in this report is actual numbers only, and does not include the expansion, which is a standard part of interpreting CWT smolt-to-adult survival data.

Table 7. Observed and estimated CWT recoveries of conventionally- and seminaturally-reared fall chinook salmon released from Forks Creek Hatchery in 1997 (BY96) and 1998 (BY97) during NATURES study.

	Raceway 21	Raceway 22	Raceway 23	Raceway 24	Raceway 25	Raceway 26
Treatment	control	seminatural	control	seminatural	control	seminatural
	$P = 0.753$					
BY96 – observed	21	18	18	24	--	--
BY96 – estimated	25	31	30	48	--	--
	$P = 0.040$					
	$P = 0.805$					
BY97 – observed	6	4	4	2	1	4
BY97 – estimated	6	4	4	4	1	4
	$P = 0.858$					

Discussion

More fish from seminaturally reared than conventionally reared groups were recaptured at the weir in all eleven trials of the Forks Creek release studies conducted between 1997-2000. However, the results were only statistically significant in three of the eleven cases. The observed differences in the recovery rates follow trends similar to those observed in previous studies by Maynard et al. (1996 a,b,d) indicating that rearing in a seminatural raceway habitat improves instream survival. Also, as noted in previous studies (Maynard et al. 1996 b,d), the skin color of the fish reared in the two treatments

was different in all years of the current study. The coloration development in seminaturally-reared salmon followed a consistent pattern from year to year. Seminatural habitat fish repeatedly developed fins lower in hue, saturation, and intensity values in culture. Examining the translation of these three color axes into a red-green-blue (RGB) color scale, the end result was for the caudal fins of seminaturally-reared fish to be greener and less bright (a darker shade) than their conventionally-reared counterparts, which were more blue in color.

In the Forks Creek experiment, the loose gravel substrate used in three earlier seminatural raceway habitat studies (Maynard et al. 1996 a,b,d) was replaced with resin rock pavers to reduce the labor involved in vacuuming decaying food and fecal material off the bottom of the raceways. Although not as easy to work as the conventional raceways, the pavers were much easier to vacuum than the loose gravel. The modified pavers also provided a similar or even more hygienic rearing environment than conventional rearing habitat. All observed pathogen outbreaks throughout four years of rearing were detected in conventional raceways. Likewise, in comparisons of general health parameters those few problems detected consistently occurred in conventional raceways. At this point in time the main improvement to the pavers would be to make them even easier to vacuum by using smaller rocks, similar in size to those used for exposed aggregate. This smaller rock would enable the wheels of the vacuum to move easily, and bring the vacuum head closer to the substrate.

Overall, the type of habitat in which fish are reared did not appear to affect their travel time. However, in all four years, release time had a significant effect on travel time. In-culture depth distribution of fall chinook salmon was also not affected by seminatural habitat. Fish in both conventional and seminatural raceways displayed similar distributions, with increasingly higher proportions of fish residing at each higher vertical location.

Whether in barren or stream-like arenas, the predator evasion ability of fall chinook salmon was improved somewhat by seminatural habitat rearing. A higher percentage of seminaturally-reared than conventionally-reared fish were able to avoid being attacked by a merganser predator during trials. It seems likely that this success stemmed from a combination of crypsis and use of shelter. Crypsis stems of course from the coloration development of the fish, and the use of shelter would be expected to occur more frequently in fish previously exposed to structure. In both arenas there were drain and influent screens behind which fish could find refuge, effectively remaining inaccessible to the merganser throughout the trial. Furthermore, the stream arena had gravel pockets, creating good hiding places for fish. It seems reasonable to expect fish, which had never had any sort of, structure in their rearing environment (conventional) to be less likely to utilize such refugia upon encounter.

The observed water quality data can be contrasted to published information of hatchery discharges (Kendra 1991). The Kendra (1991) study was based on 11 state and 9 commercial fish hatcheries in Washington sampled during the summer. Whereas data for the current study was collected during spring months. The difference in the sampling period is probably more important for assessment of the impact of hatchery discharge on

receiving streams. Effluent water quality data from Kendra (1991) are presented below:

Parameter	Minimum	Maximum	Median
Temperature	10.1	20.9	12.8
PH	6.8	9.4	7.6
Dissolved Oxygen	5.4	14.3	10.0
Total Suspended Solids	<1	9	3
Total Ammonia Nitrogen	0.02	0.89	0.20

The Forks Creek Hatchery dissolved oxygen (DO) data was higher than the Kendra (1991) data, probably because of lower ambient temperatures increasing solubility of oxygen. The lower dissolved oxygen in the effluent from the seminatural tanks may be related to additional substrate and bacterial or algae activity. For example, on 12 April 2000 the Δ DO in a seminatural rearing unit without fish was 72% of the Δ DO of a seminatural tank with fish. The addition of gravel substrate and suspended tree increases the surface area available for bacteria and algae.

The total gas pressures (Δ P) observed are probably typical for medium-sized surface water sources in Washington. Over the temperature range observed, the change in temperature (Δ T) through a seminatural rearing unit was equal to (Fig. 46; Δ T = 0.324 - 0.04T r^2 = 0.621). There was no relationship between the change in temperature through a conventional rearing unit. It is likely that a different relationship would be found if the observations were continue though the summer.

The total ammonia nitrogen (TAN) observed at Forks Creek Hatchery were lower than the data reported by Kendra (1991). The basis for the significantly higher TAN in the seminatural tank on 24 May is unknown. Compared to typical fish culture un-ionized ammonia concentrations, the values estimated at Forks Creek were very low. The significantly higher un-ionized ammonia concentration observed in the seminatural tank on 24 May was due to the higher total ammonia concentration in this tank rather the due to pH and temperature.

The Forks Creek Hatchery pH data was lower than the Kendra data (Kendra 1991) and probably related to the specific chemical composition of water supply used for Forks Creek. The total suspended solids (TSS) measured at Forks Creek are probably comparable with the Kendra (1991) data if the higher clearing data is removed.

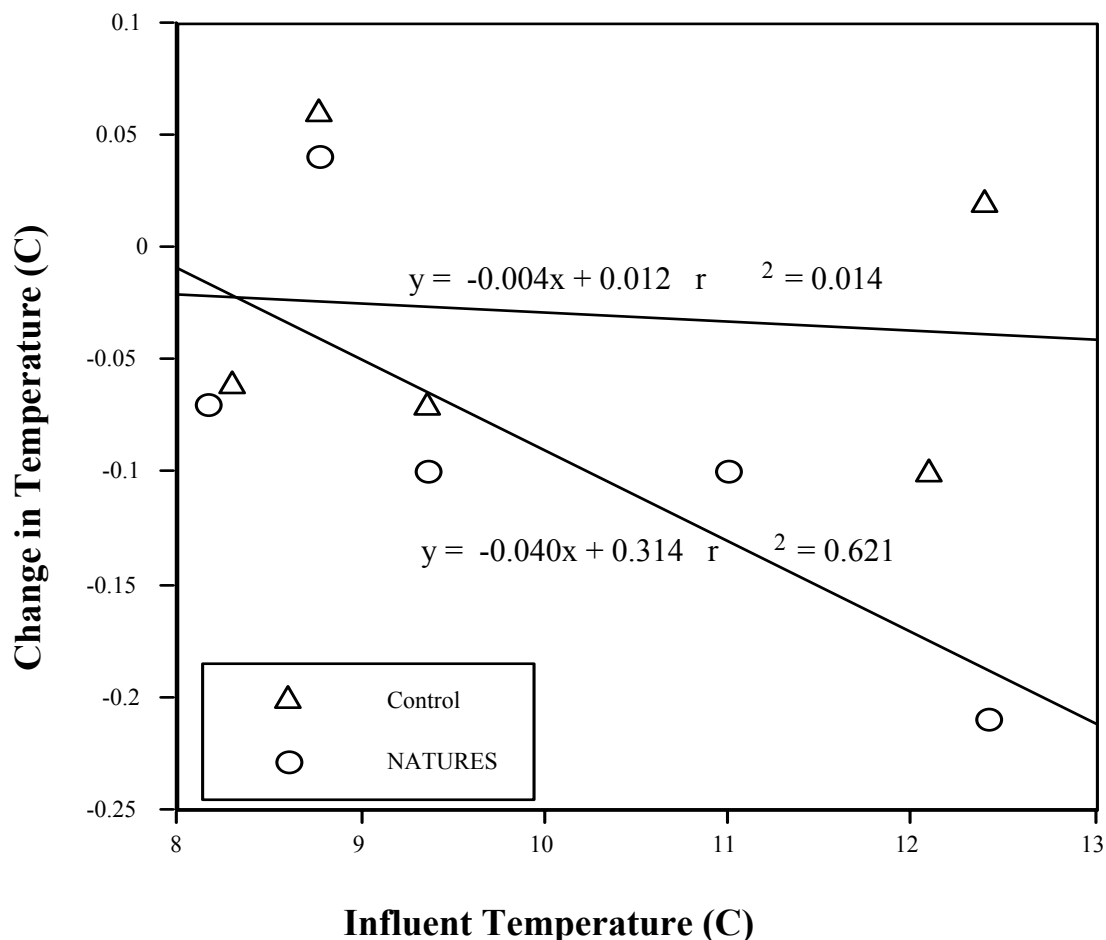


Figure 46. Change in temperature (ΔT) vs. influent temperature for seminatural and conventional rearing units.

As expected, the light levels in the seminatural rearing tanks were significantly lower than in the conventional rearing units because of approximately 60% cover with camouflage screen. The bird screening used in the conventional rearing also significantly reduced light levels to about 63% of the ambient light levels (conventional cover removed). Therefore, when discussing reductions in light levels, it is important to understand what the measured light levels are being compared to (conventional light levels with covers down or conventional light levels with covers removed). The light levels in the seminatural raceways were only 33-43% of conventional values and 21-27% of uncovered conventional raceways. Directly under the suspended trees, the light levels can be reduced by another factor of 2. It may be useful to compare these measurements with typical light levels in near-by streams.

The results of instream release and weir recovery of seminaturally and conventionally reared fish suggest that NATURES treatments can benefit cryptic coloration and instream survival at production scale and densities in a manner similar to that previously demonstrated at pilot scale (Maynard et al. 1996a,b,c,d). At this time,

only the smolt-to-adult survival of fish from the two treatments remains to be evaluated. The majority of the study fish are now at sea. As these fish return over the next several years, they should also answer the question of whether or not seminatural rearing improves smolt-to-adult survival.

Acknowledgements

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Section 2

EFFECT OF EXERCISE ON FALL CHINOOK SALMON

by

**Desmond J. Maynard, Gail C. McDowell¹, Gary A. Winans, Glen A. Snell,
Thomas A. Flagg, Conrad V. W. Mahnken, and Robert N. Iwamoto**

Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
Manchester Research Station
P.O. Box 130
Manchester, Washington 98353

¹Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon 97027

Introduction

Typically, salmonids are reared in low velocity raceways with currents less than 0.01 m/s (Pennel and McLean 1996). However, research has demonstrated that exercising salmonids by rearing them in current velocities greater than 0.05 m/s has several benefits. Exercised salmonids grow faster and have better food conversion than conventionally-reared fish (Christiansen et al. 1989, Christiansen and Jobling 1990, Christiansen et al. 1992). Regular exercise is also known to improve salmonid swimming performance (Besner and Smith 1983, Leon 1986, Schurov et al. 1986a). Most importantly, the postrelease survival of exercised fish has generally been higher than that of non-exercised fish (Burrows 1969, Wendt and Saunders 1972, Cresswell and Williams 1983, Leon 1986, Schurov et al. 1986b), with some exceptions (Lagasse et al. 1980, Evenson and Ewing 1993). The survival benefits of exercise have only been documented when rearing current velocities exceed one body length/second, for at least 1 hour a day, and for at least a 2-week period. We conducted the studies described in this section of the report to determine the effects of current velocity on fish health, growth, survival in culture and after release, morphometrics, and predator avoidance ability.

Methods

1999 Activities

The study was conducted with fall chinook salmon reared in 12 5.5-m long \times 0.6-m wide troughs, with a water depth of 0.3 m. In February 1999, fall chinook salmon from the Washington Department of Fish and Wildlife (WDFW) Minter Creek Hatchery were transported to the NMFS Manchester Research Station. The fish were divided up into six equivalent groups of approximately 10,930 and each group stocked into raceways supplied with freshwater. On 20 April 1999, 1,700 fall chinook salmon from each raceway were divided into two paired groups and placed into the 12 study troughs (850 fish per trough). One group from each pair was in an exercise treatment trough and the other group was in a non-exercise control trough (Fig. 1). Beginning on 26 April 1999, the current velocities in six troughs were increased to 0.18-0.3 m/s for 2 hours per day for 40 days. The fish in the other six troughs were not exercised and were maintained in standard single-pass raceway velocities of less than 0.03 m/s. Except for the differences in current velocity pattern, the handling and husbandry of fish in both treatments were identical and followed standard salmon culture protocols. Troughs were monitored regularly for mortalities, which were promptly recorded and removed. In-culture survival was compared using 2×2 contingency table analysis.

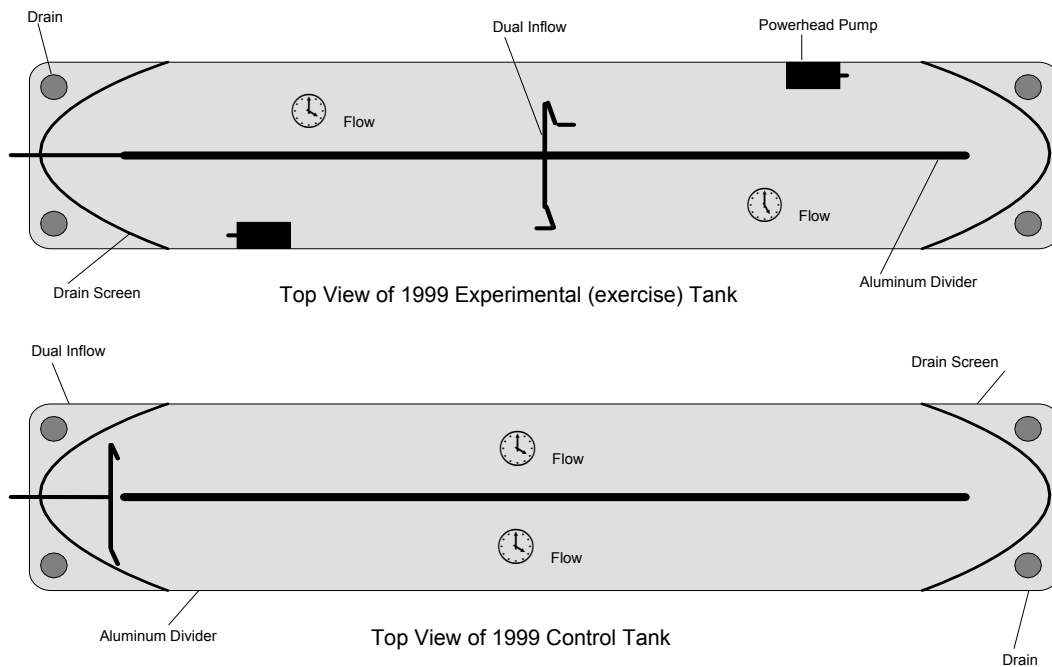


Figure 1. Schematics of exercise (top) and control (bottom) troughs used in the 1999 exercise study. Exercise troughs had centrally located flow at all times and used booster pumps to achieve exercise velocities.

The growth of fish in the two treatments was determined by removing a representative sample of 17 fish from each trough on 20 April, 17 May, and 29 June 1999. The sampled fish were anesthetized in tricaine methanesulfonate (MS 222), measured to the nearest 1 mm, weighed to the nearest 0.01 g, and then returned to the trough. A student *t*-test was then used to statistically compare the length, weight, and condition factor of the fish in the two rearing treatments.

On 29 June 1999, at least 125 fish from each trough were PIT-tagged for the instream postrelease survival evaluations. After tagging, the PIT-tagged fish were combined into two experimental and two control rearing troughs, and the remaining non-tagged experimental fish were combined into four exercise and four control rearing troughs. The fish were maintained and exercised until they were released.

The postrelease survival effect of the rearing treatments was evaluated by releasing fish from both treatments upstream of a NMFS-operated smolt collection weir on Olalla Creek in Kitsap County, Washington (47° 25' 35" N and 122° 34' 19" W). In each release, 50 PIT-tagged fish from each treatment were combined into a common hauling container and trucked to a release site (47° 27' 43" N and 122° 34' 35" W) at least 3.8 km above the weir. This release procedure was conducted a total of 15 times to produce a total release of 753 exercise and 745 control fish. The weir was checked daily beginning with the first release and tagged fish were still outmigrating throughout September 1999. The weir was removed in early October 1999 when flooding

undermined the weir. The postrelease survival of fish from the two treatments was then totaled and statistically compared with a 2×2 contingency table analysis.

On 8 September 1999, 15 untagged fish were systematically removed from each of the remaining 8 troughs for health sampling. This fish health analysis was performed using the fish health index outlined by Goede (Goede 1990). The fish were anesthetized, weighed, measured, externally examined, hematologically sampled, and then dissected for internal examination.

On 28 January 2000, a low frequency hydrophone was placed in one of the exercise troughs to examine the acoustic environment to which the salmon had been exposed. Recordings were made in three physical conditions. In one set of tests all water to the troughs and the powerhead pumps were turned off to establish ambient acoustic conditions at the facility. In another set of recordings the water was turned on, but the powerhead left off to establish the acoustic environment to which control fish were exposed. Finally the powerheads were turned on to determine how the exercise regime altered this acoustic environment. A separate set of recordings was made in a control trough under two conditions. The first recording was made with influent water running to the control trough as in normal operations. The second recording was made in the control trough while the booster pumps were running in the exercise trough, to determine how different the acoustic environments were during exercise. These recordings were analyzed with Fourier analysis software.

2000 Activities

Current velocities in 2000 were achieved differently than in years past, using a manifold water inlet system instead of booster pumps (Fig. 2). Six outlets were evenly spaced throughout the trough, three on each side of the divider, with the opening running parallel to the desired direction of water flow (a 90° ell joint at the end of a pipe). An additional two outlets dumped water perpendicularly into the trough at the end opposite the drain pipe. These two standard outlets were in use 22 hours out of the day, creating a typical single-pass flow-through scenario. The other two hours per day, the six-outlet manifold was used, spraying water from the mid-point of the water column in a recirculating pattern. The manifold system used increased water pressure to generate current velocities. The same manifold system was installed in control troughs as in exercise troughs, with an additional 90° ell joint installed at the end of each outlet, causing the water to bubble up toward the surface. With this system, the same amount of water flowed through exercise and control troughs at all times, but different flow patterns (and hence velocities) were created. Prior to placement of fish into these modified troughs, velocity measurements were made in one exercise trough to determine how high velocities could be achieved with this system. With a single trough operating, the speeds in the trough averaged 0.234 ± 0.023 m/s. This velocity was higher than under normal operating conditions because with only a single trough operating, the water pressure was higher.

On 20 March 2000, 5,000 underyearling fall chinook salmon from the WDFW Minter Creek Hatchery were systematically divided into six groups of 1,500 and placed

into the troughs. The fish in three troughs were exercised for 2 hours per day at velocities ranging from 0.09-0.19 m/s (1.75-3.62 body lengths/s at the time of measurement). The fish in the other three troughs were not exercised and were maintained in standard raceway velocities of less than 0.03 m/s, except for occasional brief periods when these control troughs were drawn down for cleaning. The treatments (exercise and no exercise) were applied for 36 days prior to the first predation bioassay trials. Except for the exercise regime and flow pattern, the handling and husbandry of fish in both treatments were identical and followed standard salmon culture protocols. Troughs were monitored regularly for mortalities, which were promptly recorded and removed. In-culture survival was compared using 2×2 contingency table analysis.

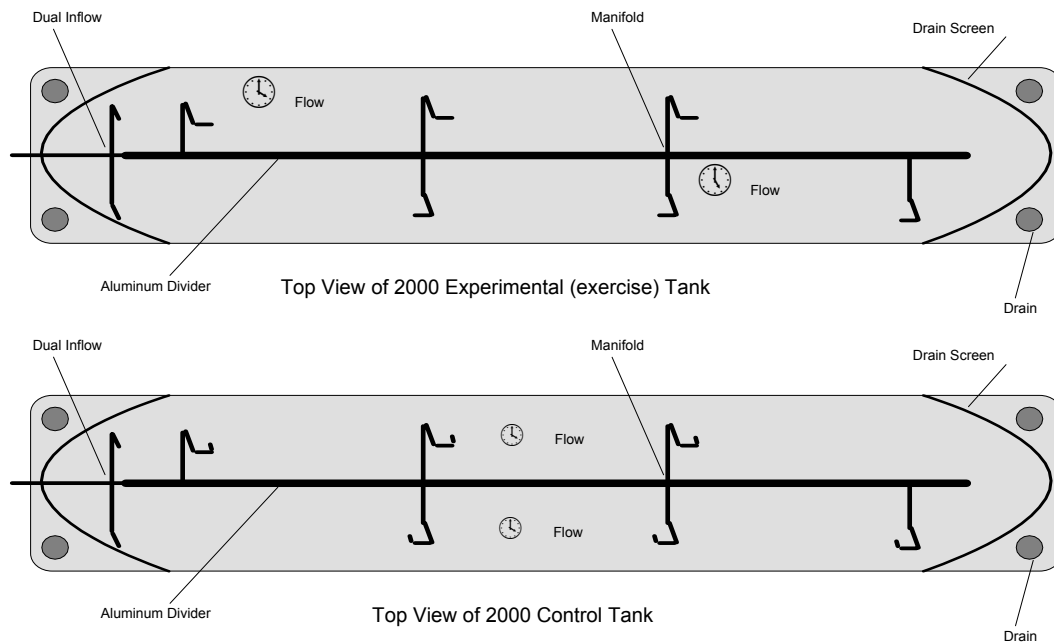


Figure 2. Schematics of exercise (top) and control (bottom) troughs used in the 2000 exercise study. Both troughs had inflow at tank head most of the time, and used inlet manifolds either parallel or perpendicular to water flow to deliver increased water flow in exercise or control environments, respectively.

The growth of fish in the two treatments was compared by removing a representative sample of 30 fish from each trough every 4 weeks. The sampled fish were anesthetized in MS 222, measured to the nearest 1 mm, weighed to the nearest 0.01 g, and then returned to the trough. Student *t*-tests were then used to compare the length, weight, and condition factor of the fish in the two rearing treatments.

On 20 June 2000, fish were size-selected from each trough. Sixty fish (79 mm in length) were removed from each trough for photonic tagging. A total of 180 fish from each treatment were tagged, with exercise fish being tagged on the right pectoral fin and control fish receiving tags on the left pectoral fin. Tagged fish were placed into separate troughs, where they received three additional days of increased water flow for 2 hours per

day. Beginning 26 June, ten tagged fish from each treatment were collected and placed into one of two 5,947-L raceways located in a fenced enclosure. The bottom of each raceway was covered with rock, which was swept up along the edges of the tank to resemble a streambed. Trials were alternated between the two arenas. Ten exercised and ten control fish were carried into the test arena and released into the downstream end (above the drain sump). Within five minutes of fish being placed in the test arena, a single hooded merganser (*Lophodytes cucullatus*) was allowed access to the arena. After twenty minutes, the bird was forced out of the arena, the tank drained, and the remaining fish removed. Fish were euthanized in MS 222, sorted according to condition (killed, live scarred, or live unscarred) and treatment (left or right pectoral fin tagged). Scarring indicated that a fish had been attacked. A total of 16 trials were run using these tagged fish. One trial had only 7 exercise and 7 control fish because of mortality in the holding tanks. When the first supply of fish was exhausted, a second batch of 50 (80-mm long) fish per trough was sorted and tagged on 28 June 2000. This time, exercise fish were tagged on their left pectoral, and control fish on their right pectoral, to control for predator visibility. An additional 10 trials were run using these fish. The performance of fish from the two rearing treatments was compared with a paired *t*-test.

A second set of predation bioassays was attempted using northern pikeminnow (*Ptychocheilus oregonensis*) as predators. As before, size-matched fish were tagged on their left or right pectoral fin by treatment. A single pikeminnow was placed in a trial arena and left overnight. Five exercise and five control fish were placed into the trial arena with a pikeminnow, and left for approximately 7.5 hours. At the end of the trial, the arena was drained, the pikeminnow removed to a holding tank, and all remaining chinook salmon sorted and tallied, as in the merganser trials. On 17 August 2000, 20 fish from each trough were systematically removed for health sampling. The fish were euthanized in MS 222 and their health compared with the Goede Index, as in 1999. Fish were again weighed, measured, externally and internally examined, and hematologically sampled, though the leukocrits were not read in 2000.

Acoustic readings were taken on 28 March 2000 to compare the differences in sound levels between exercise and control troughs. Recordings were made in an exercise trough in both exercise and non-exercise modes, as well as in a control trough in high-flow and regular-flow modes. An additional set of recordings was made and analyzed to verify reduction in sound levels from the earlier booster pump exercise method. This recording was made in a trough, configured as in 1999. These recordings were analyzed with Fourier analysis software.

The fish were examined for changes in body shape on 23 March, 25 May, and 23 August 2000. On each sampling date, 7 fish were sampled from each trough, euthanized with carbon dioxide (Alkaseltzer[™] effervescent antacid) and photographed with a digital camera while lying on their right side. Each picture included a ruler elevated to the horizontal plane of the dorsal fin. Pectoral, pelvic, and anal fins were placed into a “normal” position and held, when necessary, with dissecting needles. Red dye was brushed on the fins to highlight fin rays.

Cartesian coordinate information for 19 landmarks was collected from images

with the digitizing program tpsDig (Rohlf 1998). Thirty-five distance measurements were calculated between 15 landmarks in a truss network pattern after Winans (1984; Fig. 3). Landmarks at the anterior tip of the dorsal, anal, and pelvic fins, and at the insertion and distal point of the left pectoral fin were digitized for dorsal and anal fin height, and pelvic and pectoral fin length, respectively. Morphometric distance data were analyzed in a principal component (PC) analysis of the variance-covariance matrix using SYSTAT 10 (SYSTAT 2000). Because no size differences existed between the two groups, no adjustment was made for size-related effects. Individual PC scores were analyzed and plotted for the first major components. Fin lengths and heights were analyzed in a separate PC analysis.

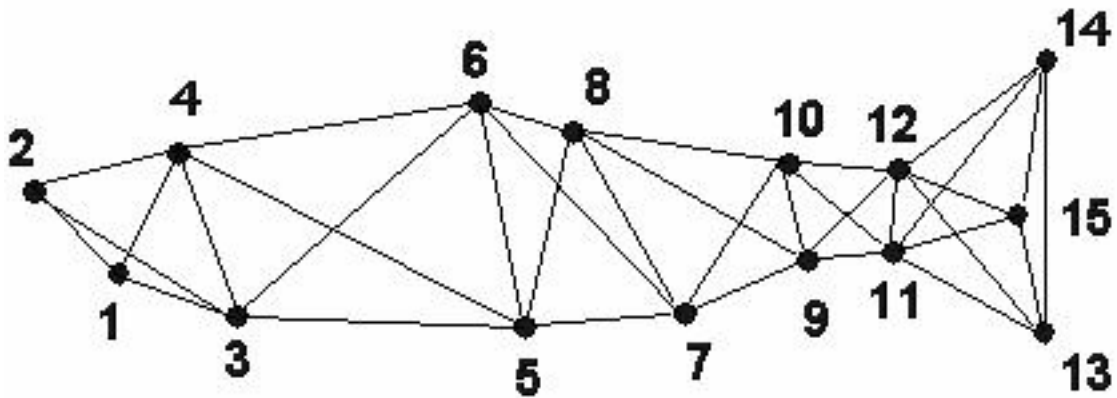


Figure 3. Location of 15 landmarks on the body outline; 35 inter-landmark distances are illustrated as solid lines. Not shown are four landmarks used to calculate dorsal and anal fin heights, and pectoral fin length.

Results

Growth

There were no significant differences in fish size or weight when they were placed into the experimental treatments on 20 April 1999 (Figs. 4 and 5) or in May after a month of rearing. However, at PIT tagging on 29 June 1999, after two months of rearing in the experimental treatments, the exercise fish had become significantly longer than the unexercised controls. The average weight of fish in both treatments was still nearly identical in this June sampling. Mean lengths and weights were similar in the two treatments in September 1999 (Figs. 4 and 5). There were no significant effects of current velocity on body size at any sampling point in 2000 (Figs 6 and 7).

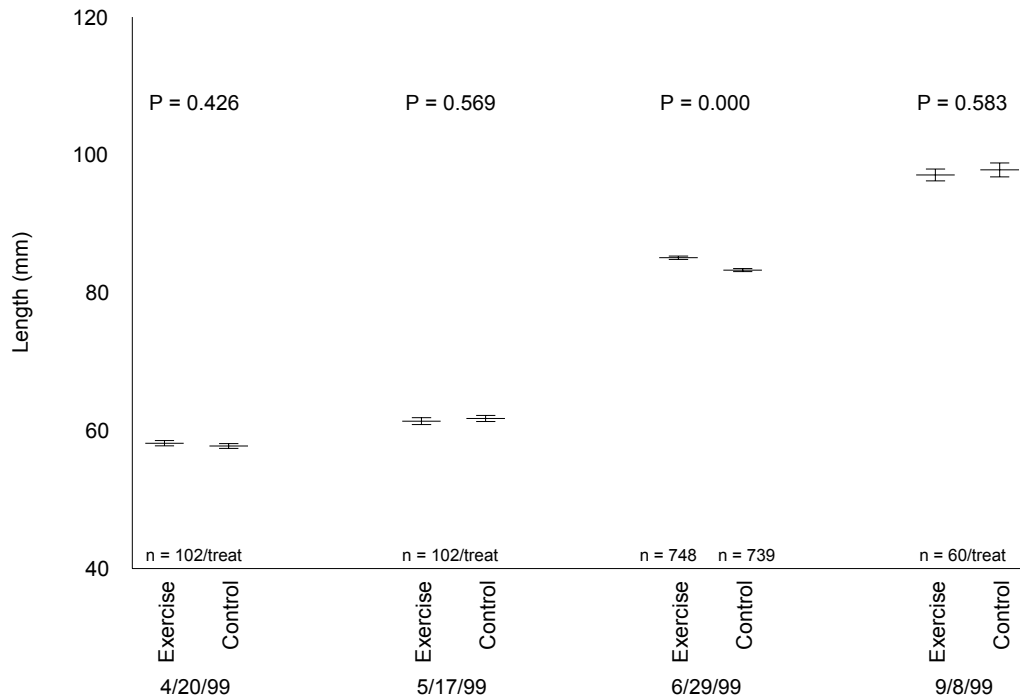


Figure 4. Mean lengths (\pm S.E) of fall chinook salmon throughout rearing in exercise or control troughs in 1999. P values are based on *t*-tests.

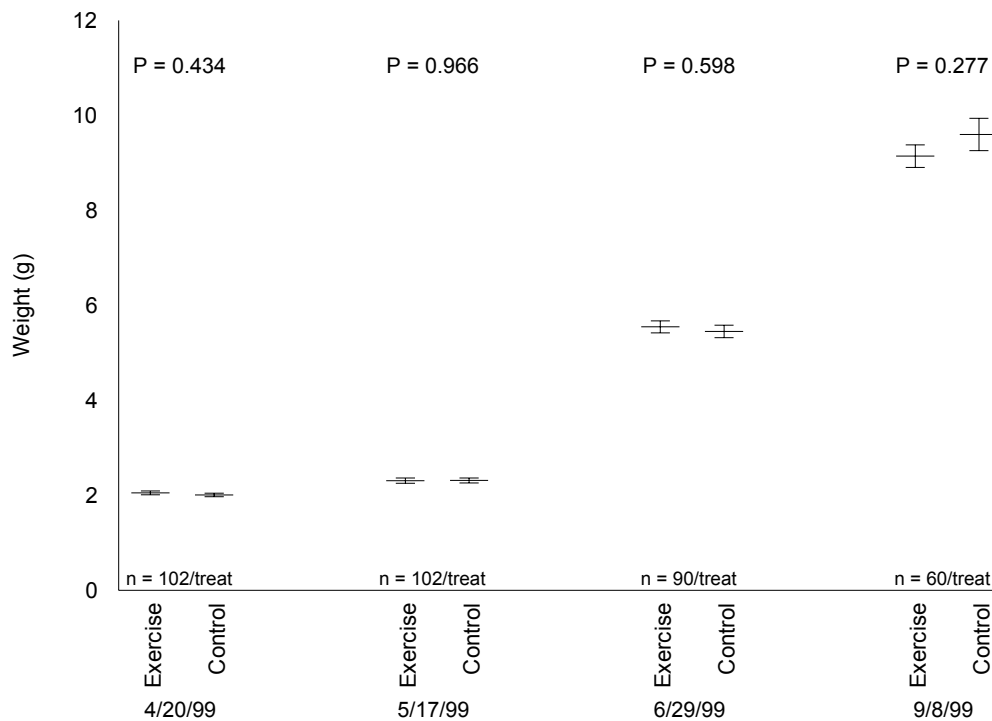


Figure 5. Mean weights (\pm S.E) of fall chinook salmon throughout rearing in exercise or control troughs in 1999. P values are based on *t*-tests.

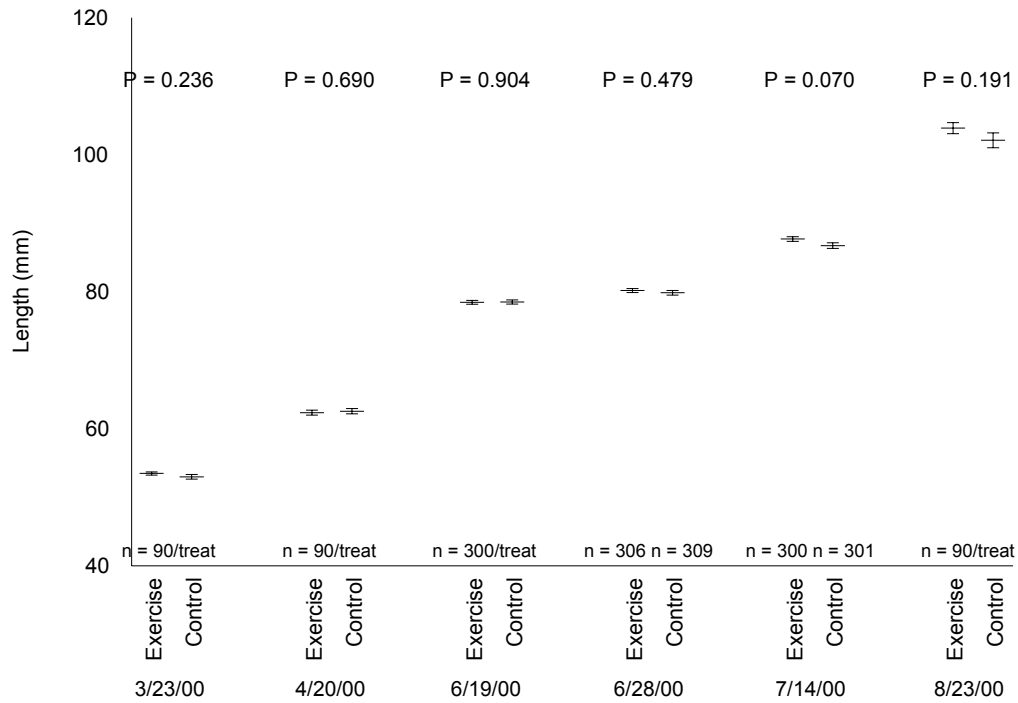


Figure 6. Mean lengths (\pm S.E) of fall chinook salmon throughout rearing in exercise or control troughs in 2000. P values are based on *t*-tests.

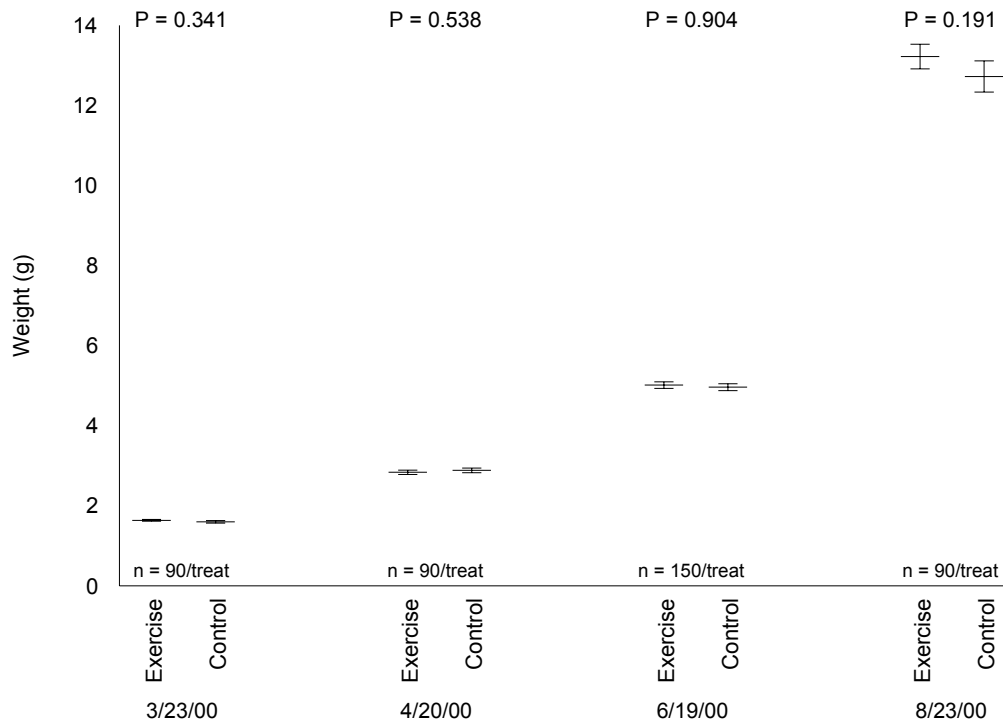


Figure 7. Mean weights (\pm S.E) of fall chinook salmon throughout rearing in exercise or control troughs in 2000. P values are based on *t*-tests.

In-culture Mortality

In 1999, only 0.5% of the exercised and 0.6% of the control fish had died ($P = 0.592$) by June (Fig. 8). Cumulative mortality was significantly higher ($P = 0.020$) in control than exercise troughs by 10 August 1999. The exercise program was immediately suspended and mortality rates of the controls remained higher than that of exercised fish. The exercise program was resumed after main cause of the mortality (Ich, *Ichthyophthirius multifiliis*) the Ich problem had been successfully treated with formalin baths. The experiment was fully terminated when a massive mortality spike occurred just prior to fish health sampling. The spiked nature of this event suggests it was driven by an unobserved water supply event (e.g., a transient interruption of water flow or environmental toxin) rather than a pathogen. There was no indication fish dying during this event were diseased. In-culture mortality in 2000 did not differ significantly through initiation of predator avoidance bioassays (Fig. 9). However, control fish had suffered significantly higher mortalities by the time fish were diagnosed with Furunculosis (*Aeromonas salmonicida*) on 31 July 2000. At this time, fish were treated with 2% medicated Terramycin (TM-100) feed for 10 days. Fish were diagnosed with *Ichthyophthirius* on 31 August 2000, and treated with formalin baths on 5, 12, 19, 22, 25, and 29 September 2000. Fish were re-diagnosed with Furunculosis on 19 October 2000, and treated with 4% medicated TM-100 (1% ration) for 10 days, starting 30 October 2000.

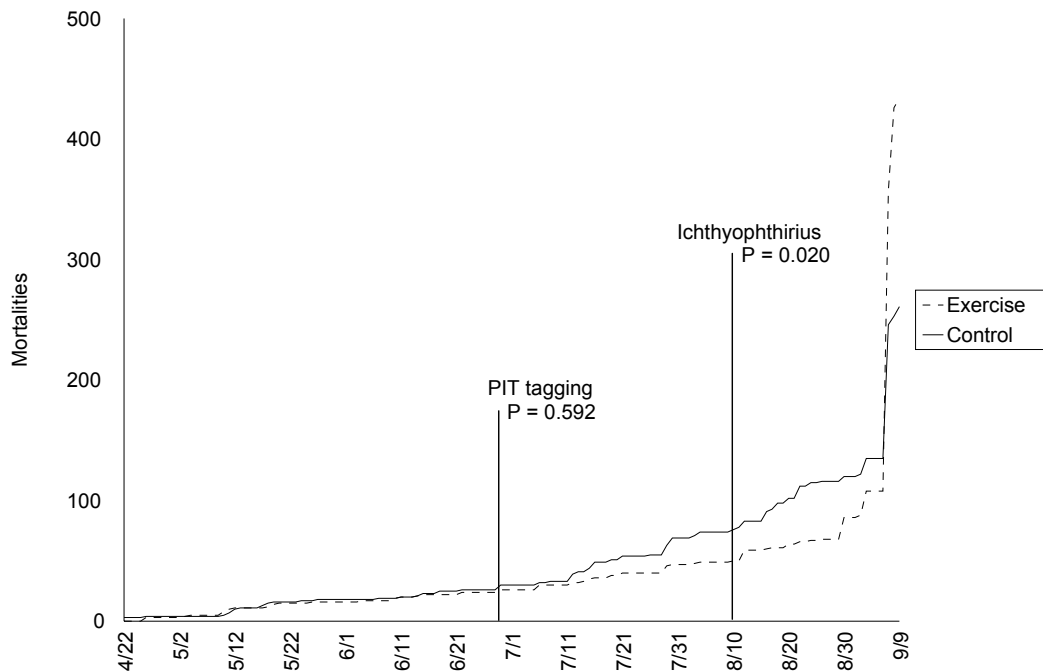


Figure 8. In-culture mortality of fall chinook salmon reared in exercise or control troughs from initiation of experiment through release in 1999. P values are based on contingency table analysis

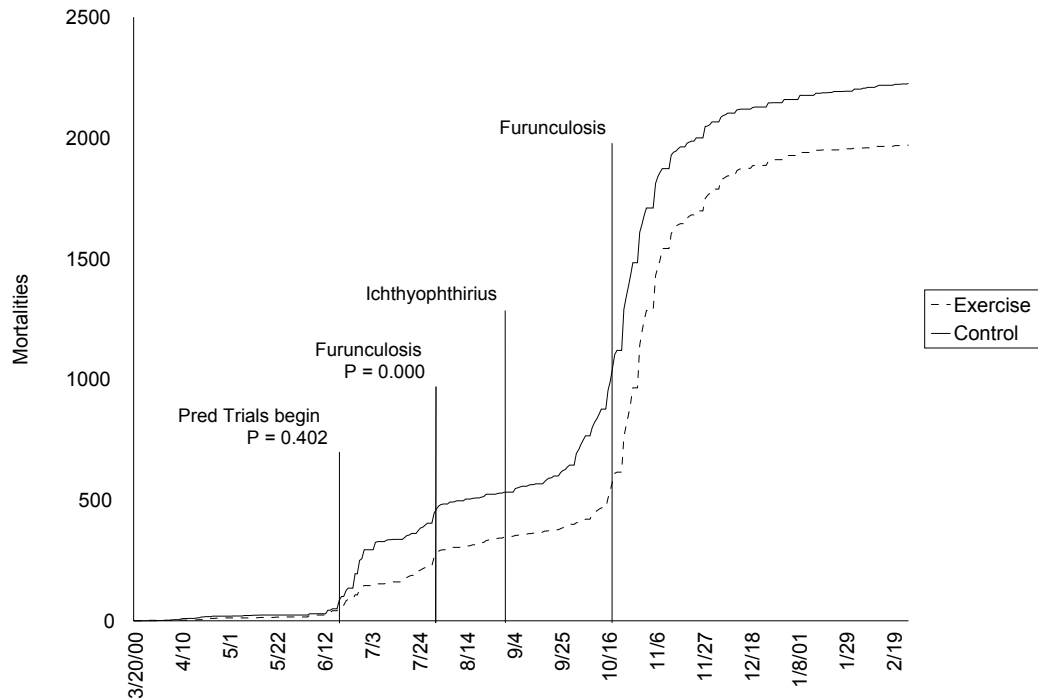


Figure 9. In-culture mortality of fall chinook salmon reared in exercise or control troughs from initiation of experiment through release in 2000. P values are based on contingency table analysis.

Postrelease Survival

Although some fish at release were observed to be better able to hold position against the current than others, the downstream travel time of fish in both treatments was similar (Figure 10). Significantly ($P = 0.000$) more control (34.8%) than exercised (23.5%) fish were recovered at the wier (Fig. 11). Unfortunately, no study fish were collected in the stream sections electrofished in September, 1999.

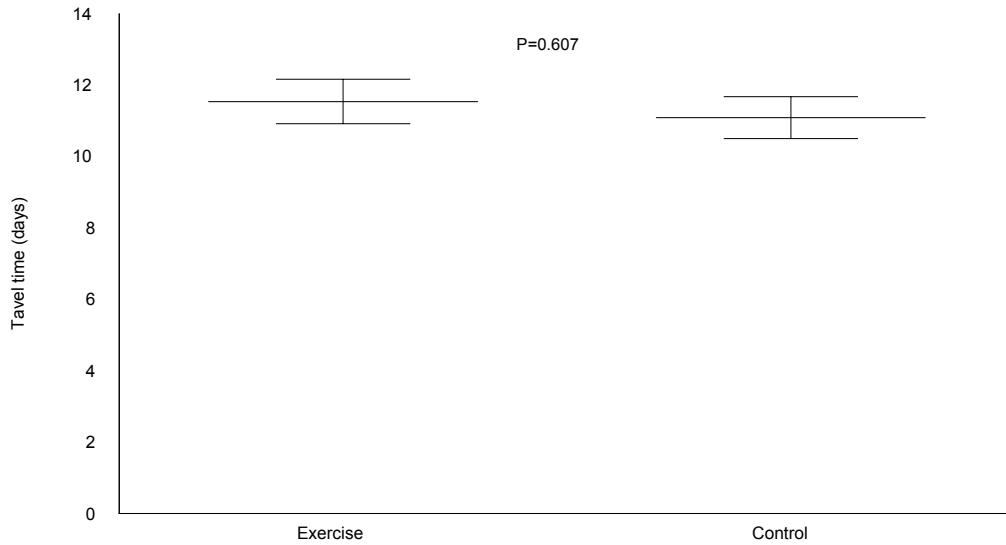


Figure 10. Mean traveling time \pm standard error (time from release to recapture at weir) for fall chinook salmon reared in exercise (n = 177) or control (n = 260) troughs in 1999. P values are based on t-tests.

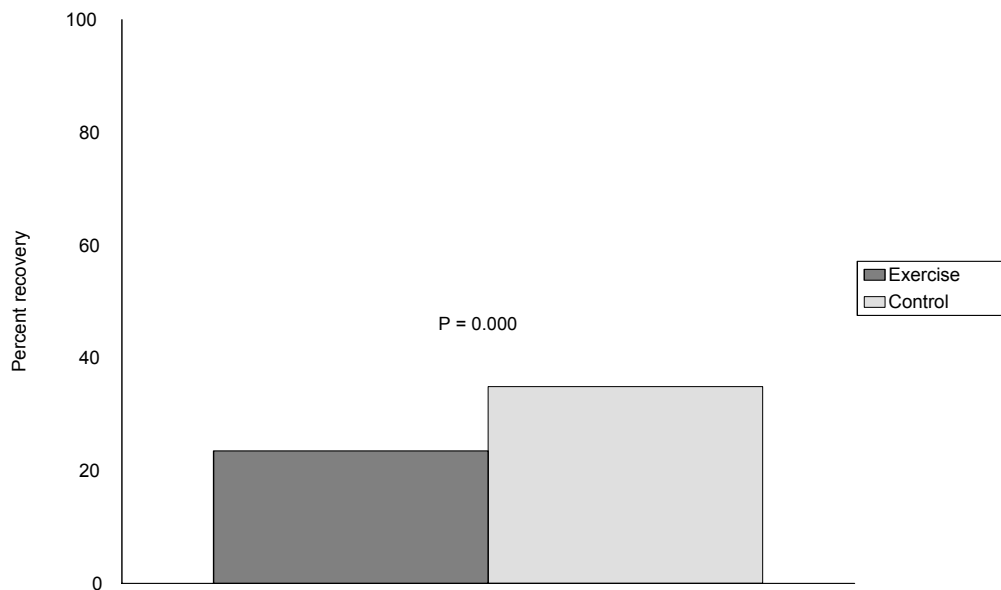


Figure 11. Percent recovery of fall chinook salmon reared in exercise or control troughs and released into Olalla Creek in 1999. Probability values (P) are based on contingency table analysis.

Predator Avoidance Bioassays

No fish (control or exercise) were preyed upon by the predator (as determined by absence of scarring on any fish when all remained alive throughout the trial) in 5 of 26 predator avoidance bioassays using mergansers. In 8 trials, the merganser scarred fish but killed none.

Only 10.1% of control fish and 9.3% of exercise fish were attacked by the predator ($P = 0.873$, Fig. 12).

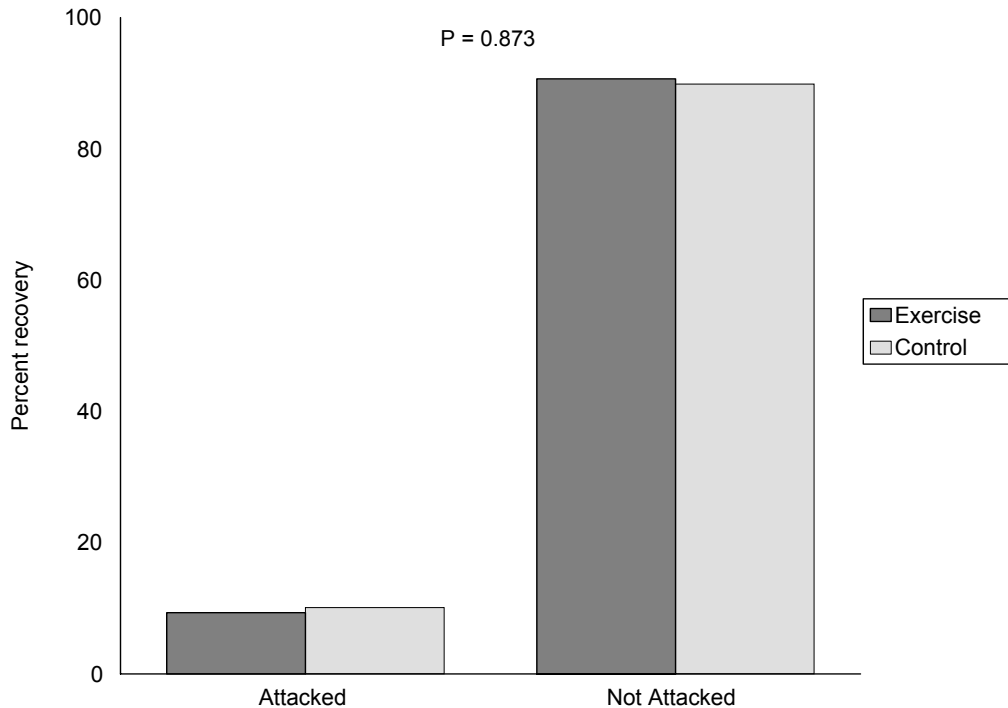


Figure 12. Predator avoidance, expressed as percentage, of exercise and control fall chinook salmon in 2000 predation bioassays ($N = 257/\text{treatment}$). P value is based on a paired t -test.

Fish Health

On 8 September 1999, after the fish had been out of the exercise program for about a month, both treatments displayed statistically similar occurrence of fin erosion, with over half of each treatment having mildly eroded fins (Fig. 13). Fish in both treatments had excellent eye condition and near perfect opercle condition with no statistically significant differences occurring between the treatments for these two parameters. Only 75% of the control and 68% of the exercised fish had normal gills, but there were again no statistically significant ($P = 0.275$) differences between the two rearing treatments. The pseudobranchs of all the fish in both treatments appeared normal and problem free. There was no difference in mesentary fat content ($P = 1.000$). There

were enlarged spleens in three exercised fish, and none in control fish, but differences were not statistically significant. All the fish in both treatments had a hindgut classification of 0 indicating no problems. There were significantly more class C livers in the control than exercise fish ($P = 0.004$), where more class B livers were found. Livers are categorized by color, with B being light red, and C being creamy in appearance. Bile and kidney condition of the two treatments did not statistically differ ($P = 1.000$). As expected, the sex ratio of fish in both treatments was similar ($P = 0.855$) with 46.7% of the controls being males and 45% of exercised fish being males. The hematocrit, leukocrit, and plasma protein values of exercised salmon were all significantly ($P = 0.027$, $P = 0.014$, $P = 0.031$, respectively) higher than that of the control fish (Fig. 14).

Fish health sampling took place on 17 August 2000, after 67 days of exercise (Fig. 15). As in 1999, the fins of most fish in both treatments displayed some erosion ($P = 0.355$). All fish sampled had normal eyes, opercles, and pseudobranchs. Almost all fish from both treatments had normal gills, with only 1.7% of control fish and 3.3% of exercise fish displaying any problems ($P = 0.998$). Mesentary fat classes were similar ($P = 0.780$), with the vast majority of the fish in both treatments in the intermediate classes. Spleens and hind gut condition were identical between treatments, with 100% of both groups having normal spleens and no hind gut inflammation. Liver classification was very similar ($P = 1.000$) for both groups, with 100% of control and 98.3% of exercise fish having class C livers. The bile of fish from the two treatments differed significantly ($P = 0.009$). Exercise fish had a higher occurrence of class 1 and 2 bile scores. Kidney conditions were similar ($P = 0.648$), with only 5% of control fish and 3.3% of exercise fish having problematic kidneys. There were more males in the exercise than control fish (63.3% versus 50%), but differences were not statistically significant ($P = 0.141$). The hematocrit of exercise fish was significantly higher ($P = 0.018$) than that of control fish (Fig. 16), but plasma protein values did not differ significantly ($P = 0.665$).

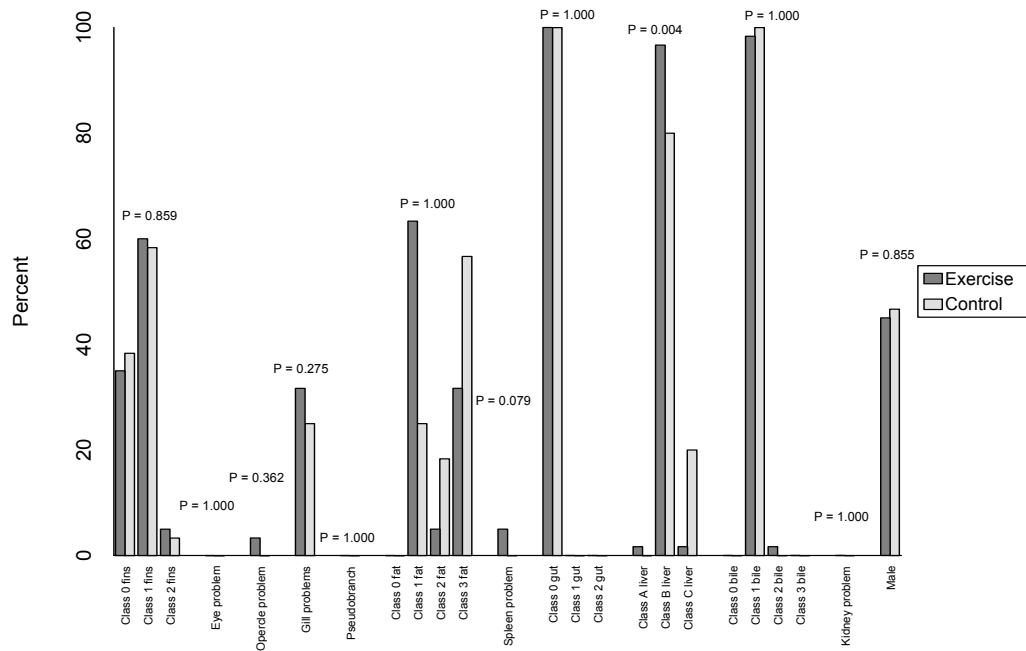


Figure 13. Percentage of fish in different classes or displaying problems in 1999 fish health sampling, using a modified Goede Index. P values are based on contingency table analysis.

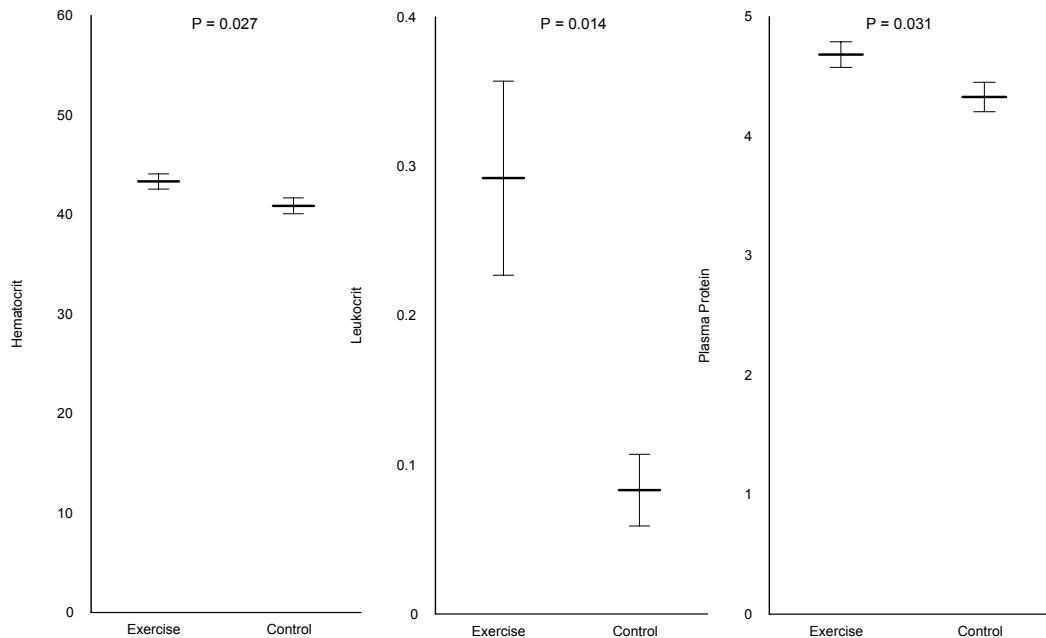


Figure 14. Means (\pm S.E) of hematocrit, leukocrit, and plasma protein values from fall chinook salmon reared in exercise or control troughs in 1999. P values are based on *t*-tests.

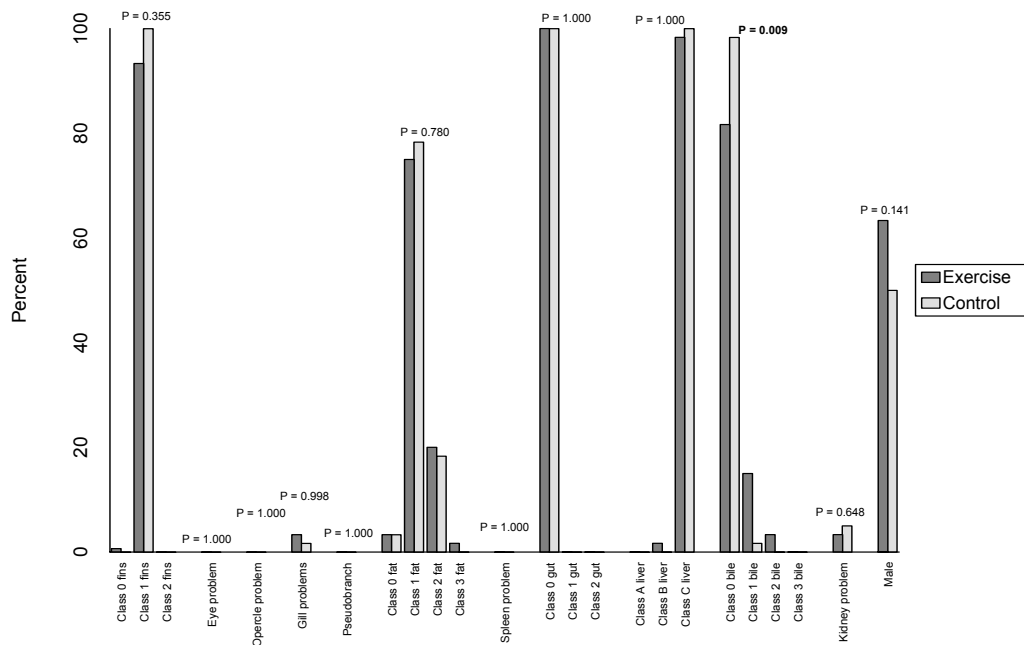


Figure 15. Percentage of fish in different classes or displaying problems in 2000 fish health sampling, using a modified Goede Index. P values are based on contingency table analysis.

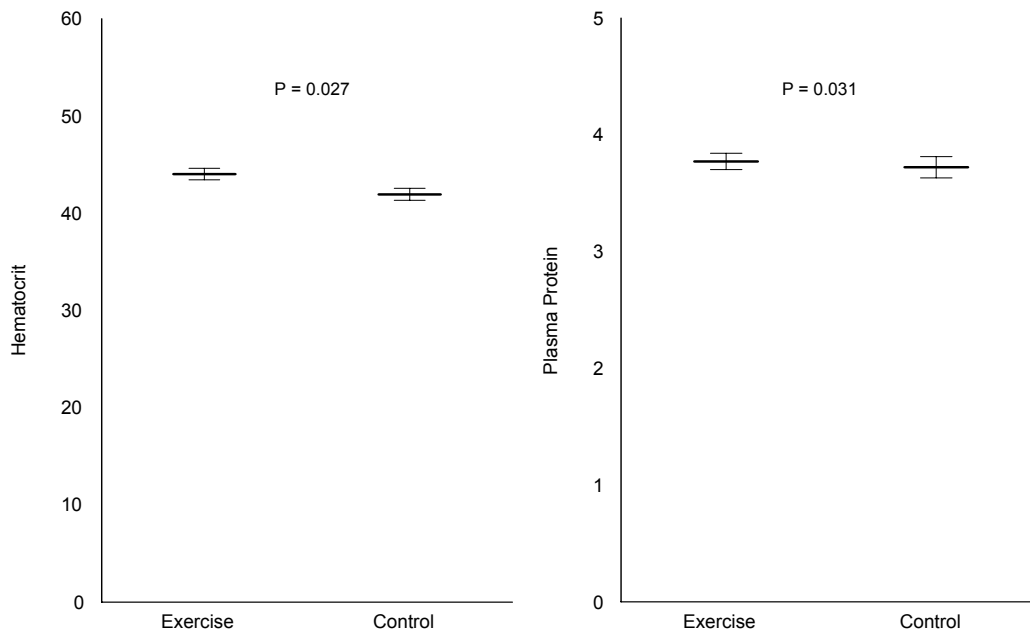


Figure 16. Means (\pm S.E) of hematocrit and plasma protein values from fall chinook salmon reared in exercise or control troughs in 2000. P values are based on t -tests.

Acoustics

Measuring sound levels in a trough with booster pumps off and no water running to the tank determined ambient sound levels in 1999. These measurements indicated that ambient sound levels at the Manchester Research Station were higher than anticipated (Fig. 17). This spectrograph shows that ambient levels were steady and fairly low at most frequencies above 400 Hz. Even below 400 Hz most sound was created at the harmonics of 60 Hz. Most non-harmonic levels occurred below 200 Hz. Turning water on to the trough increased the acoustic level, masking some of the 60 Hz harmonics with higher levels at a broader range of frequencies, up to about 600 Hz. Finally, turning on the booster pumps to produce exercise currents further increased underwater sound levels. Most of the increase in noise below 400 Hz occurred at the 60 Hz harmonics. Above 400 Hz, the increase in volume occurred at every frequency. The maximum amplitude difference between the two spectrographs is 10 decibels.

The exercise and control troughs had different water influent plumbing in 1999, which was a source of sound differences between treatments at all times. Sound levels were measured in each trough with the water running, but no booster pumps on. Subtracting the amplitude of the exercise trough spectrograph from the amplitude of the control trough spectrograph generated a comparison (Fig. 19). The control trough, with water falling perpendicularly into the trough, was consistently louder at almost all frequencies than the exercise trough, with water entering the water column at a 45 degree angle. Thus, during non-exercise times, exercise fish were actually exposed to lower sound levels than control fish.

In 2000, the inflow during non-exercise times was identical, and no sound level differences occurred during normal operation. In the range below 300 Hz, the exercise troughs were roughly 3-10 dB louder at most frequencies, but above 300 Hz, the control troughs were predominately about 5 dB louder than exercise troughs (Fig. 20).

The difference in amplitudes between high flow and normal flow in the exercise troughs was of slightly greater magnitude at lower frequencies (Fig. 21). The greatest amplitude difference below 300 Hz was roughly 18 dB, with the exercise trough ranging from -5 to 18 dB louder in exercise mode. Most noise occurred at a broad range of frequencies below 90 Hz, all at 10 dB or more. The difference between high and normal flow modes in the control trough was similar (Fig. 21), but with less broad-range noise below 90 Hz.

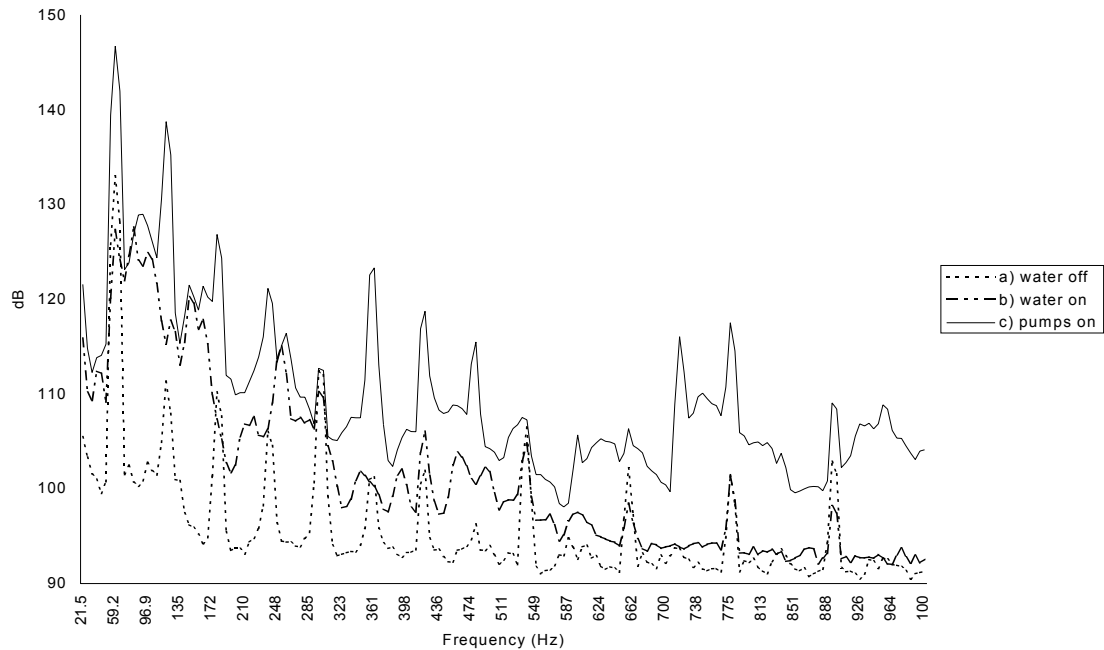


Figure 17. Acoustic measurements (20-1000 Hz) in 1999 exercise trough with a) water and pumps off (ambient levels), b) water on and pumps off, and c) water and pumps on.

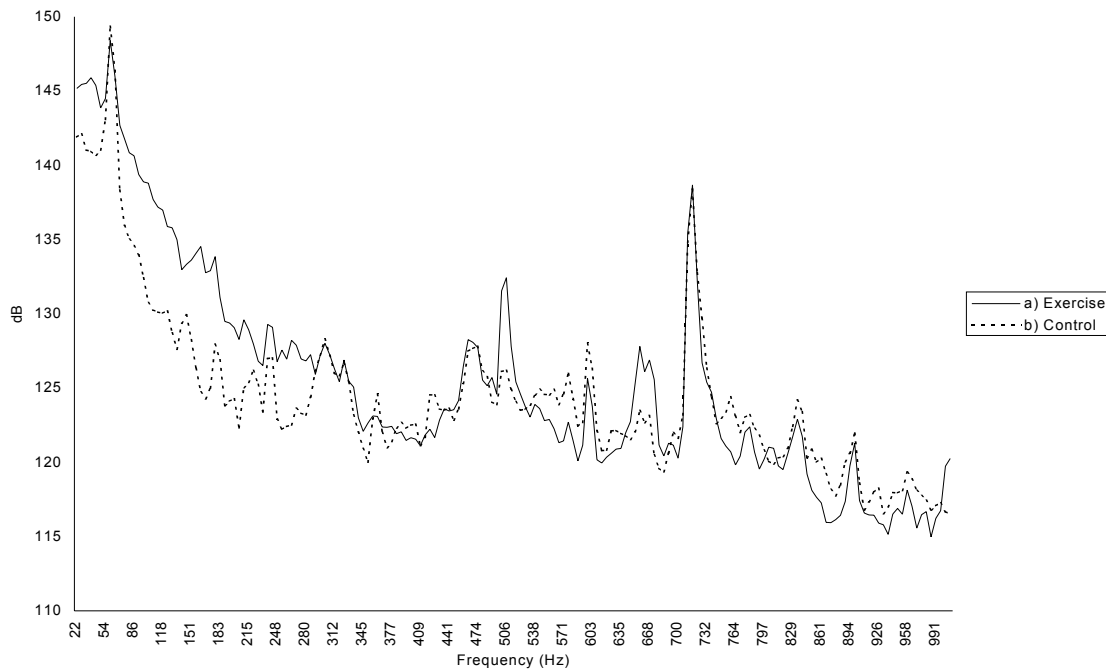


Figure 18. Acoustic measurements (20-1000 Hz) in 1999 with exercise booster pumps running. Values are for a) exercise trough with pumps running, and b) adjacent control trough during exercise.

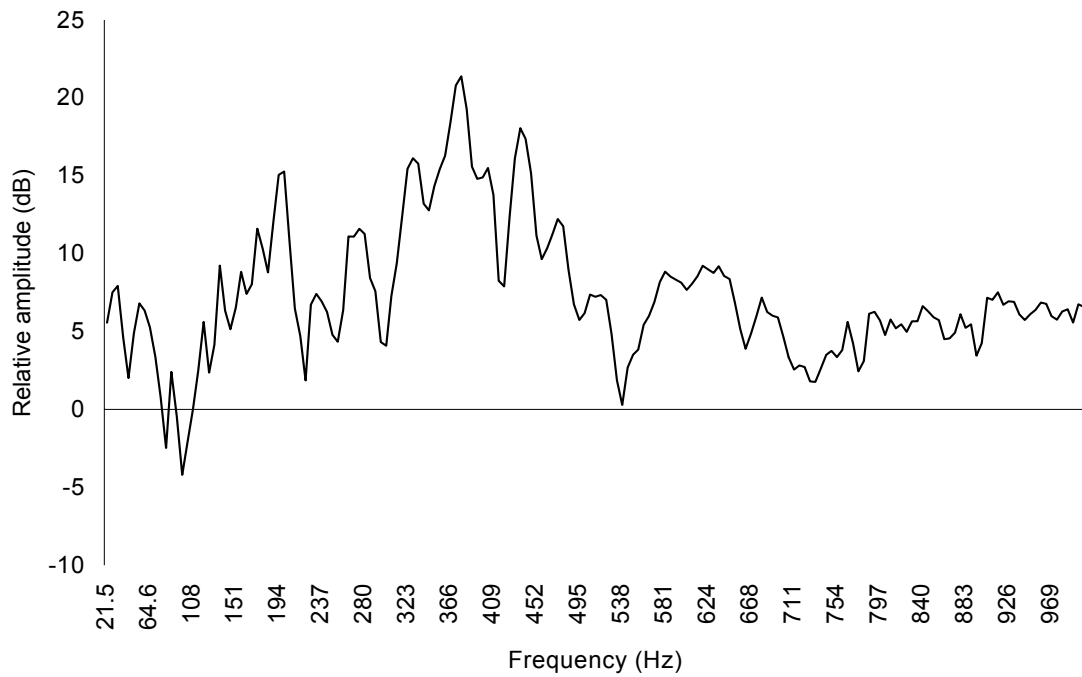


Figure 19. Difference in influent acoustic levels (20-1000 Hz) in 1999. Values are control trough minus exercise trough amplitudes.

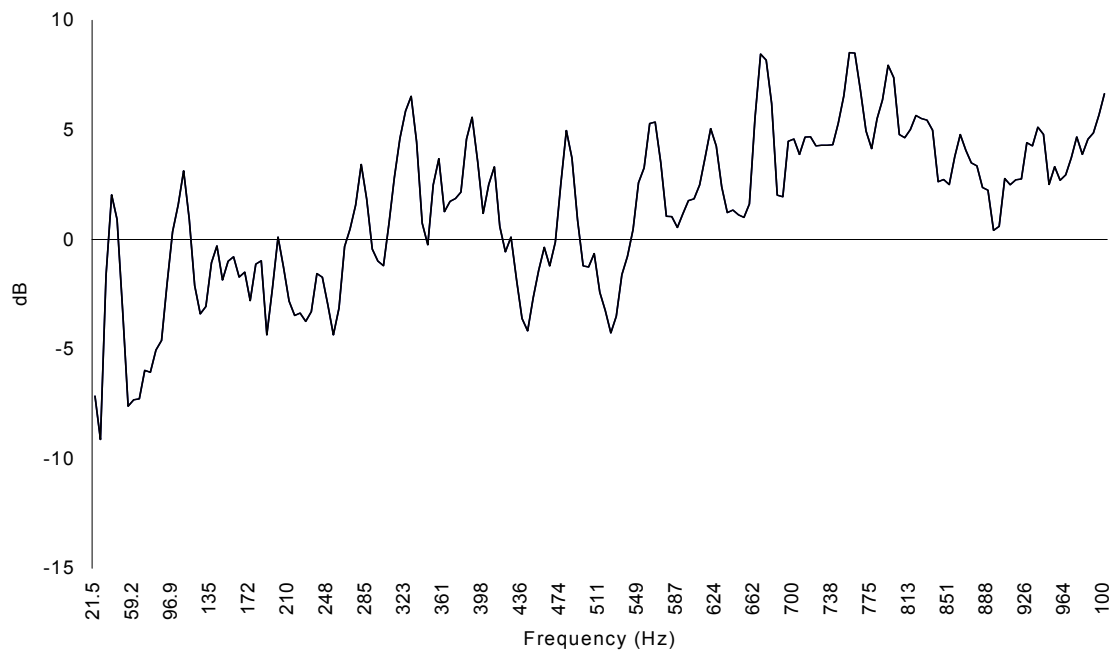


Figure 20. Difference in acoustic levels in high flow mode (20-1000 Hz) in 2000. Values are control trough minus exercise trough amplitudes.

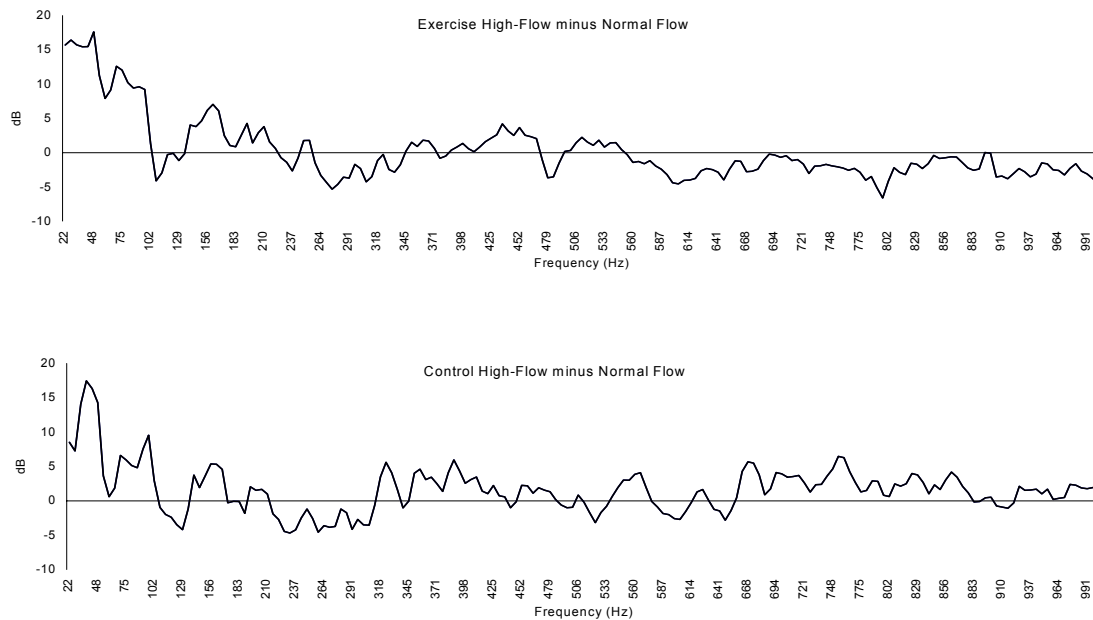


Figure 21. Difference in acoustic levels in exercise (top) and control (bottom) troughs (20-1000 Hz) in 2000. Values are high flow minus normal flow amplitudes.

Morphometrics

Univariate Analyses

There were no significant univariate differences between the control and exercise groups at any time period for any of the characters we examined, including: body weight, fork length (distance 2-15), the four fin heights and lengths, any of the individual tail measurements, and body depth at head (distance 3-4), dorsal fin (distance 5-6), and tail (distance 11-12).

Multivariate Analyses

The PC analysis of fish in March, prior to experiment initiation, did not reveal any statistically significant differences along any of the first five PC axes. Differences were seen in the May PC analysis of the control and exercised fish after 35 days of exercise. The first five PC's explained 86% of the total variance. PC1 was a size variable: all measurements had positive loadings (appendix) and PC1 scores were correlated to fork length ($r = 0.98$). No significant differences between the treatment groups were seen along PC2. Factor scores for PC3 were significantly different between the control and exercised fish ($t = 2.68$, $P_{40} = 0.045$). Important morphometric characters, as indicated by large component loadings on PC3, are indicated in Figure 22 (see also appendix). Exercised fish had a larger ventral lobe on the caudal fin, as well as a slightly larger forehead (distance 2-4), and correspondingly smaller measurements in the region

between the head and dorsal fin. PC3 scores are plotted in Figure 23. It is noted that none of the first 5 PC's resembled the elongated caudal peduncle change (re. distance 11-12 and distance 9-11) associated with smoltification (Winans and Nishioka, 1987). No differences were noted at PC4 or PC5.

In August, after 71 days of exercise, the two groups were again examined. There were no univariate or multivariate differences detected. Most remarkably, no multivariate component resembling PC3 (described above) was observed in the PC analysis. There were no differences found between the treatment groups in any PC analysis of fin heights (dorsal and anal) and fin lengths (pectoral and pelvic) at any time period.

We conclude that a slight, but statistically significant morphological response to current was seen within a month in young-of-the-year chinook salmon. Body shape changes included an enlarged caudal fin and shortened mid-body. Our ability to evaluate this response was questionable in the same group of fish 2 months later because the fish were treated for Furunculosis and removed from the exercise regime.

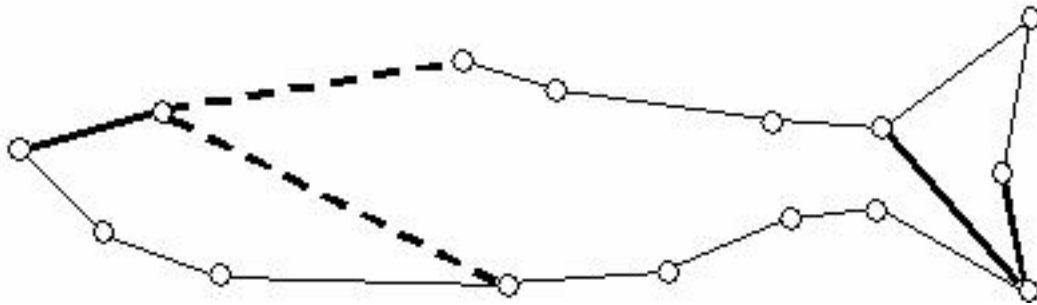


Figure 22. Important morphometric distances in principle component (PC) analyses of 35 distance characters (illustrated above) for PC3. Weighting coefficients for these characters are + (solid lines) or - (dashed lines). Body outline is included for illustrative purposes.

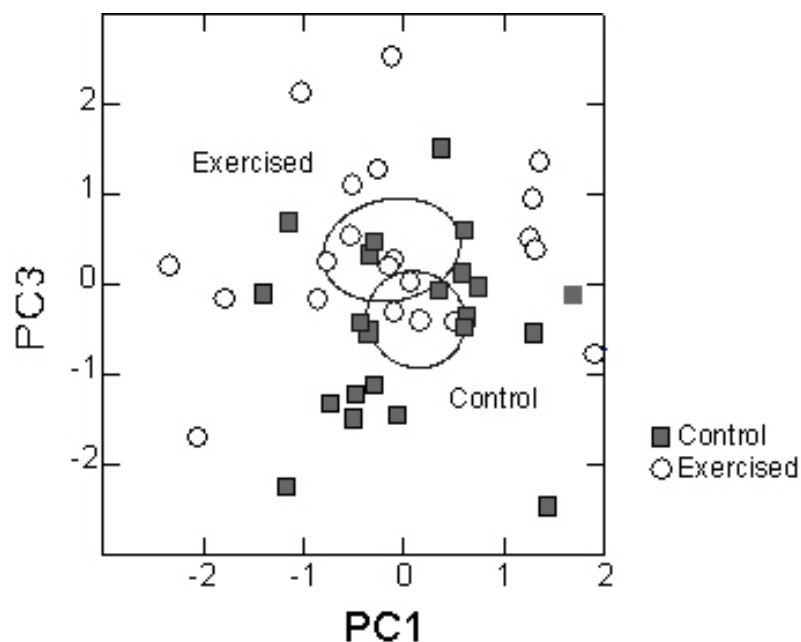


Figure 23. Distribution of factor scores on PC1 vs. PC3. 95% confidence ellipses for the sample centroid are included per treatment group.

Discussion

The powerhead exercise protocol tested appeared to have some moderate benefits and significant biological costs for fall chinook salmon. As reported for other species (Davison and Goldspink 1977, Leon 1986, East and Magnan 1987, Houlihan and Laurent 1987, Christiansen et al. 1989, Josse et al. 1989, Christiansen and Jobling 1990, Farrell et al. 1990, Christiansen et al. 1992, Young and Cech 1993, Young and Cech 1994 a and b, Jarboe and Grant 1996, Jorgensen et al. 1996), this specific exercise protocol seemed to improve the growth performance of fall chinook salmon. East and Magnan (1987) reported that exercise increased the mass of the fish, but not their length. Research with other species indicates that exercised salmonids exhibit a different growth hormone profile than unexercised fish, which seems to explain the enhanced growth performance of exercised fish (Barrett and McKeown 1988). The growth performance advantage conferred upon our exercised fall chinook salmon appeared to be lost after the fish were removed from the exercise program for 4 weeks. It should be considered that other factors, such as the experimental changes at tagging and the pathogen outbreak may also have been responsible for the elimination of this growth advantage.

In 1999, there was some evidence that exercise provided the fish with enhanced disease resistance. The *Ichthyophthirius multifiliis*-induced mortalities were higher in the control tanks. Thus suspending the exercise protocol was a successful tactic for reducing Ich-related loss. In 2000, the control fish suffered higher losses.

The consistent differences in fish health in both 1999 and 2000 were likely a direct result of exercise. Exercise is known to improve physiological condition of organ systems related to swimming in other fish species (Sanger 1972, Davison and Goldspink 1977, Farrell et al. 1990). It is also known that exercised fish have higher levels of protein synthesis involved in muscle building (Houlihan and Laurent 1987). It is possible the increased plasma protein level observed in the exercise treatment fish is related to this increased protein synthesis. It is not as clear why exercise increased leukocrit levels.

With more control than exercised salmon reaching the weir, the 1999 experiment failed to demonstrate that exercise increases the postrelease survival of fall chinook salmon. It is unclear why moderate exercise seem reduced the number of fall chinook salmon collected at the wier. We speculate that control fish may have been less able to hold position in stream currents and were therefore flushed rapidly downstream to the weir. If so, large numbers of control fish would have been recovered in a very short period of time. Concurrently, exercised fish would remain in the stream and be susceptible to predation. Contrary to our speculation, however, mean travel time in the 1999 study was almost identical between treatments. Late August through mid September is not the typical outmigration window for Minter Creek fall chinook salmon, which are normally released from the hatchery in May. Hence, it is possible that an increased number of fish took up residence in the stream, and remained to outmigrate as yearlings.

In a similar study with Atlantic salmon Shurov et al. (1986b) observed similar results with far fewer exercised Atlantic salmon being recaptured at the weir because they were able to hold position in the stream. They concluded exercise increased survival. However, lacking instream residence data, we cannot draw such a conclusion, and must instead conclude that fewer exercised fish reached the weir because fewer of them survived downstream migration. Given the importance of being able to partition survival between residents and migrants, we recommend future exercise studies be conducted where both populations can be sampled in such a manner as to provide a robust analysis.

It is possible the loud sound produced by the powerheads damaged the lateral line or inner ear hearing capabilities of the exercised salmon. Salmonid sound detection involves both the ear and lateral line (Popper and Carlson 1998). These two sensory systems serve independent functions, but both use the same basic information – sound waves. Some literature suggests that salmonids have nearly constant frequency-sensing thresholds between <1 and 150 Hz. Then, the thresholds rise sharply for higher frequencies, with near total loss of detection at >380 Hz (Knudson et al. 1992, Kalmijn 1988). Studies of the lateral line indicate that it is responsive to frequencies from 1 to 345 Hz, but that maximum sensitivity is between 10 and 170 Hz (Weber and Schiewe 1976). Also, “sound must be at least 10 dB more intense than background noise to be detected” (Popper and Carlson 1998). In terms of damage that may be done to fish hearing systems by auditory bombardment, Popper and Carlson (1998) note “high-intensity sounds (180-200 dB re: 1 μ Pa) might not affect hearing generalists such as the oscar and salmonids”. In addition, Popper and Carlson observed, “Loss of sensory hair cells in the lateral line, if it occurs as a result of sound damage, could degrade schooling behavior of fish as well as their detection of predators and prey within short distances.”

In the 1999 study, the sound levels produced by the booster pumps created a 17.85 dB increase in amplitude at the first harmonic of 60 Hz (120 Hz), in comparison to the control troughs. Most of the largest amplitude differences occurred at the 60 Hz harmonics. Comparing control troughs to exercise troughs in non-exercise mode, the style of inflow used in the control troughs generated much more sound than that used in the exercise troughs. This is significant to discussion of environmental differences in 1999, because while booster pumps were only on for two hours a day, the water flowed differently to exercise versus control tanks 24 hours a day. While the differences in these two methods was not as severe as pumps on versus off (topping out around 15 dB relative amplitude), this was a constant, steady bombardment of control fish at practically all frequencies in salmonid hearing range.

The alternative approach used in 2000 to generate exercise currents successfully reduced underwater sound levels in both the exercise and control troughs. In 1999 exercise troughs, the booster pumps generated signals at amplitudes of 120-150 dB below 800 Hz, whereas the manifold system had sound levels of 95-130 dB during exercise currents. The average reduction in amplitude across all frequencies between 20 and 800 Hz was 23.27 ± 0.37 dB. There was a similar reduction in the control troughs, comparing the volumes transmitted to the control troughs with booster pumps on in 1999 to the increased flow manifold method in 2000. This amounted to an average amplitude reduction of 20.92 ± 0.36 dB in the control troughs.

Also of interest in 2000 were the inter-treatment differences in underwater sound levels. A comparison of control and exercise troughs in high-flow mode showed that the manifold system used in the exercise troughs had an amplitude on the order of 5 to 10 decibels higher than control troughs at frequencies below 275 Hz. However, at frequencies above 275 Hz, the manifold in use in the control troughs had higher amplitude, on the order of 5 to 10 dB. The end result was that during the non-exercise periods (22 hours per day) the sound levels in tanks of both treatments were identical, and sound levels during high-flow periods were similar to one another. It is not clear at this time if the magnitude of these differences is small enough to conclude that this manifold exercise system does not induce sound-related treatment differences.

There was no visible predator avoidance benefit to the exercise system tested in 2000. Unexpectedly, the merganser used for predation bioassays attacked very few fish. Upon completion of the merganser bioassays we initiated another round of bioassays using northern pikeminnow, in the hope of finding a more successful predator. The pikeminnows, however, failed to attack even a single chinook salmon and therefore were not an effective trial predator. So, while the merganser trials showed no significant benefit for exercised fish, we feel that this is an inconclusive result.

In summary, the exercise protocol tested in 1999 provides minimal growth and fish health benefits for fall chinook salmon and a possible risk of reduced postrelease survival. We believe pathogen-related mortality was reduced by exercising the fish for only part of the day and suspending the exercise program when pathogen induced mortalities first appeared. The system developed in 2000 also improved the inculture survival associated with the exercise regimen. The 2000 system failed to confer a

statistically significant predator avoidance advantage to exercised fish compared to non-exercised controls. Perhaps, with further research, exercise protocols will evolve that produce greater biological benefits than costs for fall chinook salmon.

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Appendix

Component loadings on principal axes 1-5. "Important" characters are bolded in PC3.

	1	2	3	4	5
Distance2_4	0.412	0.298	0.536	0.093	0.114
Distance3_4	0.707	0.055	-0.016	-0.026	0.133
Distance1_3	0.295	-0.084	-0.072	-0.018	0.328
Distance1_2	0.358	0.218	0.243	-0.004	0.001
Distance2_3	0.675	0.122	0.159	-0.001	0.218
Distance1_4	0.432	-0.109	0.215	0.128	0.346
Distance4_6	1.266	0.316	-0.337	-0.222	-0.367
Distance5_6	1.019	-0.119	-0.163	0.184	0.135
Distance3_5	1.670	0.066	-0.256	-0.085	-0.120
Distance4_5	1.977	0.260	-0.470	-0.193	-0.179
Distance3_6	1.364	-0.010	-0.060	0.058	-0.054
Distance6_8	0.382	0.153	0.010	0.175	0.053
Distance7_8	1.008	-0.181	0.018	0.062	0.124
Distance5_7	0.520	0.025	0.248	0.225	-0.061
Distance6_7	1.321	-0.086	0.018	0.243	0.110
Distance5_8	0.790	-0.052	-0.017	0.162	0.121
Distance8_10	1.062	-0.299	0.262	-0.690	0.234
Distance9_10	0.392	-0.207	-0.070	0.328	-0.075
Distance7_9	0.437	-0.782	0.068	-0.009	-0.166
Distance8_9	1.160	-0.833	0.152	-0.033	-0.032
Distance7_10	0.793	-0.122	0.048	-0.247	0.120
Distance10_12	0.252	-0.079	-0.283	0.456	0.284
Distance11_12	0.250	-0.037	-0.055	-0.004	0.141
Distance9_11	0.249	0.491	0.023	-0.076	0.085
Distance10_11	0.421	-0.163	-0.143	0.438	-0.088
Distance9_12	0.386	0.449	-0.059	-0.085	0.404
Distance12_14	0.641	0.236	0.296	0.179	-0.364
Distance14_15	0.533	0.129	0.305	0.021	-0.040
Distance13_15	0.401	0.038	0.464	-0.047	-0.147
Distance11_13	0.586	0.366	0.232	0.109	0.200
Distance12_15	0.339	0.054	0.200	0.115	-0.255
Distance12_13	0.707	0.202	0.432	0.097	-0.185
Distance11_15	0.344	0.127	0.103	0.083	0.136
Distance11_14	0.804	0.250	0.250	0.127	0.038

Variance Explained by Components

1	2	3	4	5
22.898	2.597	1.588	1.470	1.239

Percent of Total Variance Explained

1	2	3	4	5
66.348	7.525	4.602	4.260	3.591

Section 3

EFFECTS OF SEMINATURAL HABITAT REARING ON COHO SALMON, 2000-2002

by

Desmond J. Maynard, Geraldine E. Vander Haegen¹ John E. Colt,
Gail C. McDowell², and Thomas A. Flagg

Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
National Marine Fisheries Service
2725 Montlake Boulevard East
Seattle, Washington 98112

¹Washington Department of Fish and Wildlife
600 Capitol Way North
Olympia, Washington 98501-1091

²Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon 97027

Introduction

New salmon culture techniques must be developed if the survival of hatchery coho salmon (*Oncorhynchus kisutch*) recruiting to the fishery or spawning population is to be increased. Public hatcheries produce up to 80% of the salmon available for harvest and are often the only broodstock source available for restoring depleted natural runs. Lower than optimal survival rates force hatcheries to rear and release more fish per recruit to the fisheries. Fortunately, there appears to be scope for improvement as the smolt-to-adult survival of hatchery fish is often much lower than that of wild-reared salmon. It appears that salmonids produced with traditional fish culture techniques lack many of the behavioral, physiological, and morphological characteristics needed to survive in the wild immediately after release (see the review by Maynard et al. 1995). It may be possible to promote the expression of these wild characteristics by rearing salmonids in a hatchery environment that resembles natural stream and river habitats. This is the paradigm behind the NATURES seminatural raceway habitat concept.

The National Marine Fisheries Service (NMFS) and Washington Department of Fish and Wildlife (WDFW) have been cooperatively developing a Natural Rearing Enhancement System (NATURES) consisting of seminatural raceway habitat, live food diets, exercise systems, predator avoidance training, and underwater feed delivery systems (Maynard et al. 1995, Maynard et al. 1996a,b,c,d, Maynard and Flagg 2001, Maynard et al. 2001 a,b). The most successful component of NATURES rearing has been the development of seminatural raceway habitat. Seminatural raceway habitat is composed of gravel substrates, inwater structure, and overhead cover that are installed in raceways to produce a rearing environment that resembles the stream and river habitats utilized by juvenile chinook and coho salmon. This differs from the environmental enrichment research previously conducted with coho salmon, which included structure and cover, but lacked substrate (Vander Haegen and Appleby 1998). Studies conducted with chinook salmon (*O. tshawytscha*) have shown that seminatural raceway habitat rearing can improve instream postrelease survival up to 50% (Maynard et al. 1996c). The following study was initiated to determine if seminatural raceway habitat rearing produces similar increases in coho salmon postrelease survival (Maynard et al. 2001b). BPA participation was focused on coordinating information transfer of NATURES variables developed under BPA funding to the experiment. This report focuses on the progress of the study through 2002.

The study's experimental design tests the hypothesis that rearing coho salmon in seminatural raceway habitat increases their smolt-to-adult survival. The experimental approach is to rear coho salmon in standard-sized control and seminatural raceways for at least the last two months of culture, release them into the wild, and then compare their smolt-to-adult survival. A relatively unique attribute of the study is that it is being conducted with paired raceways at five Puget Sound hatcheries spread over a large geographic range. This approach enables the findings to be extrapolated throughout western Washington and removes the specter of the findings being unique to a single facility.

Project Objectives

1. Starting with designs used in previous experiments, construct and install NATURES rearing habitat that is compatible with daily activities at a salmon hatchery.

2. Compare cryptic color development of fish reared using conventional techniques to the color development of fish reared using NATURES habitat.
3. Compare growth of fish reared using conventional methods to fish reared using NATURES habitat.
4. Compare the health of fish reared using conventional methods to fish reared using NATURES habitat.
5. Compare the smolt-to-adult survival of fish reared using conventional methods to fish reared using NATURES habitat.

Approach

Study Sites and Experimental Design

The research is being conducted at WDFW Kendall Creek Hatchery near Kendall (WA), Soos Creek Hatchery near Auburn (WA), Minter Creek Hatchery near Purdy (WA), Sol Duc Hatchery near Sappho (WA), and Issaquah Hatchery near Issaquah (WA). Seminatural raceway habitat research was initiated in the first three hatcheries during the 1999 broodyear experimental period, with Issaquah and Sol Duc hatcheries being added into the study during the 2000 broodyear experimental period. The ponds at Kendall, Soos Creek, and Issaquah Hatcheries are standard raceways that are about 3 m wide by 30.5 m long, while Minter Creek Hatchery has much larger raceways that are about 6.1 m wide and 36.6 m long. The rearing vessels at Sol Duc Hatchery are large operational Burrow's ponds. At Kendall Creek Hatchery, Soos Creek Hatchery, Issaquah Hatchery, and Sol Duc Hatchery the fish are reared in first-pass water, while at Minter Creek Hatchery they are reared on aerated second-pass water. There are marked differences in the distance the fish must migrate downstream to reach the estuary. At Minter Creek Hatchery, the fish are released almost directly into the estuary, while at Kendall Creek, Soos Creek, Issaquah, and Sol Duc Hatcheries they are released many kilometers inland. Land use along the banks of these migratory corridors also markedly differs. The fish released from Kendall Creek and Sol Duc Hatcheries migrate down medium size rivers that pass through rural, agricultural, and forest land corridors. In contrast, the fish released from Soos Creek and Issaquah Hatcheries primarily migrate through suburban-urban corridors. In addition to these differences, the Issaquah coho salmon smolts must migrate through a large lake before reaching Puget Sound via the Lake Washington Ship Canal. These differences enable the study to determine if the NATURES seminatural raceway habitat concept can be usefully employed over a large array of geographic and hatchery conditions.

At each facility, two similar raceways were selected for the experiment. One of these serves as an unmodified control, while the second is fitted with seminatural raceway habitat. The fish are conventionally-reared until their second year of life when the experimental fish are introduced into the raceways equipped with seminatural raceway habitat for final rearing. A control and experimental group of coho salmon will be reared at each of the five facilities through 2004 to provide a total of 18 paired releases for experimental evaluation of smolt-to-

adult survival.

It was necessary to refine previous seminatural raceway habitat components for installation in the production raceways at each WDFW facility prior to initiating the experiment. The first step was to develop a concrete gravel paver that was lower in cost, more durable, and easier to install than the resin-rock pavers used in an earlier study at the WDFW Forks Creek hatchery with chinook salmon (Maynard et al. 2001b). The new paver was fabricated by first covering the bottom of a 46 by 46-cm mold that is 5 cm deep with 2.2-cm gravel similar in color to the stream and river bottoms where the fish are released. Concrete (colored to match river sand) was then poured in the mold and allowed to cure. After curing, the pavers were removed from the mold and stored on pallets until installation. The pavers were installed by simply laying them down side by side until the entire raceway bottom was covered. Paver weight alone is sufficient to hold them in place. A brick saw is required to cut pavers into smaller pieces to fill in gaps along the raceway wall edge. Using this technique, pavers were successfully installed at all five hatcheries. In addition, uncolored concrete pavers (20.5 cm wide by 41 cm long by 3 cm thick) were installed over the control pond bottom at Sol Duc Hatchery because the concrete aggregate was exposed.

Camouflage net covers were developed, constructed, and installed at all the hatcheries. As in previous seminatural raceway habitat studies, military camouflage netting is suspended within 90 cm of the water surface. The netting is suspended from aluminum frames and covers from 50 to 80% of the raceway surface. The camouflage net frame design is similar at four of the hatcheries, but different at Minter Creek Hatchery because of longer side span of its double width ponds. The design used at Issaquah Creek, Kendall Creek, Sol Duc River, and Soos Creek hatcheries is a rectangular 1.67 by 3.1-m frame constructed from 2.5-cm square aluminum tube. The frame is attached by pins to a galvanized piece of 10 by 10-cm angle iron to form a hinge. The angle iron is then bolted to the concrete wall on one side of the raceway. When lowered, the opposing frame end is supported by a 2.5 by 2.5-cm piece of angle-aluminum attached to the other raceway side. Two hydraulic lift struts are attached to the cover frame to reduce the effort required to lift the frame to the fully open position. This design enables fish culturists to quickly and easily open the covers when they need to feed fish or vacuum the raceways. The Minter Creek Hatchery covers are built from 5-cm square aluminum tube and are 6.4 by 6.4-m squares that span the raceway. Each aluminum frame is supported by wheels that rest on a track running the length of the raceway. Stainless steel cable spans the frame diagonally for extra support, and the camouflage net was initially draped over these cables and fastened to the cover frame. However, after the covers failed due to snow load the camouflage net was suspended below the frame on wire ties that should break away when loaded with snow. There are three covers per raceway and each cover can be moved back and forth on the rail as necessary for raceway vacuuming.

The instream structure used in this experiment is similar to that used in previous NATURES research. At all hatcheries structure has been installed by suspending a stainless steel cable the length of the raceway and hanging denuded fir trees weighted with rebar from that cable. Trees are attached to the cable with carabiner-type clips and can be readily removed for cleaning or replacement. This structure differed from that used in previous WDFW research, where plastic containers were sunk to the raceway bottom with sandbags (Vander Haegen and

Appleby 1998).

In the BY 1999 experimental rearing period, the fish were transferred into the NATURES raceways as soon as modifications were completed. Both control and experimental fish were maintained in similar conditions until this time. After being placed into the experiment, the fish were reared similarly except for the presence or absence of seminatural raceway habitat.

In 2002, BY 2000 fish were transferred into the NATURES raceways when they were coded-wire tagged, when no other rearing space was available, or when modifications were completed. Both control and experimental fish were maintained in similar conditions until this time. After being placed into the experiment, the fish were reared similarly except for the presence or absence of seminatural raceway habitat.

Experimental Rearing

BY 1999 Experimental Rearing Period

The control and experimental raceways at Kendall Creek Hatchery each received approximately 51,000 BY99 coho salmon on 11 January 2001. Of the fish in the control raceway, 7,656 were coded-wire tagged and adipose fin-clipped and an additional 7,656 were coded-wire tagged alone. The seminatural raceway had 7,656 coded-wire tagged and adipose fin-clipped fish, as well as 7,658 coded-wire tagged only fish. These fish were tagged on 28 June 2000, prior to initiation of experimental rearing.

Soos Creek Hatchery was the second facility to begin NATURES rearing in 2001. On 25 January, approximately 55,000 BY99 coho salmon were transferred into the seminatural raceway at Soos Creek Hatchery. In both the control and experimental raceways, 5,100 fish were coded-wire tagged and adipose fin-clipped, with an additional 5,100 fish coded-wire tagged only. All tagging was completed prior to initiation of the experiment.

Minter Creek Hatchery was the last facility to initiate experimental rearing. Coded-wire tagging took place prior to experimental rearing, on 21 and 22 June 2000, when 10,040 of the coho salmon designated as controls received coded-wire tags and adipose fin clips, and 10,124 of those designated to be NATURES fish received coded-wire tags and adipose fin clips. The control raceway at Minter Creek Hatchery initially contained 298,200 BY99 coho salmon, and 300,075 coho salmon were transferred into the seminatural raceway on 28 February 2001.

BY 2000 Experimental Rearing Period

BY 2000 coho salmon were placed in the control and experimental raceways at Kendall Creek Hatchery beginning in July 2001. On 2 and 3 August 2001, the coded-wire tagged fish were loaded into the raceways as they were tagged. Each raceway received approximately 51,400 BY2000 coho salmon. A total of 20,064 fish from the conventional raceway were coded-wire tagged (half of them adipose clipped), and a total of 20,007 fish from the seminatural raceway were coded-wire tagged (half of them adipose clipped). Roughly 30,000 coho salmon, which were adipose clipped but not coded-wire tagged, were also put into each raceway.

Sol Duc River Hatchery coded-wire tagged BY00 coho salmon into the conventional and seminatural raceways on 12 December 2001. For the conventional raceway, 12,629 coho salmon were coded-wire tagged and adipose clipped; 12,533 were coded-wire tagged only; and an additional 64,900 were adipose clipped only, and received no coded wire tag. The seminatural raceway received 12,805 coho salmon with coded-wire tags and adipose clips; 12,691 coded-wire tagged only fish; and an additional 64,900 adipose-clipped only coho salmon.

At Minter Creek Hatchery, 283,600 BY00 coho salmon were placed into experimental seminatural raceway habitat on 13 December 2001, of which a total of 20,320 fish were coded-wire tagged. A total of 283,600 (20,294 coded-wire tagged) conventionally-reared fish were placed into the matching control raceway on 21 December 2001.

Soos Creek coho salmon were placed into the seminatural raceway the second half of December 2001, during coded-wire tagging. Of those reared in the conventional raceway, 20,899 were coded-wire tagged, while 20,885 of those reared in the seminatural raceway were coded-wire tagged.

Issaquah Hatchery had 25,012 coho salmon tagged for controls and 25,514 tagged for seminatural rearing. Tagging took place 27 February through 1 March 2002. Fish were first placed into the experimental rearing habitat after coded-wire tagging.

Growth, Coloration, and Health

BY 1999 Experimental Rearing Period

At all experimental sites a sample of 100 fish was removed monthly from each raceway, weighed (to the nearest 0.001 g), measured (fork length to the nearest 1 mm), and means compared with *t*-tests. At least 30 fish in each sample were photographed with 400 ASA color slide film using a Nikon 8008S single lens reflex camera equipped with a micro lens (60 mm) and circular polarizing filter. The camera was mounted on a photographic light stand equipped with two quartz halogen lamps (300 W). The light was filtered through photographic gel to simulate daylight.

Before being photographed, the fish were anesthetized in tricaine methane-sulfonate (MS 222) solution in black dishpans, and then placed individually on a clear acrylic angled stand over a standardized blue background. The fish were photographed at least twice.

Each photograph was mounted in a standard plastic slide mount and placed on a PVC plate (with the center drilled out) attached to the stage of a stereoscopic binocular microscope. A fiber-optic light illuminated the slide from below. The image was then recorded by a Hyper HAD RGB color video camera, captured, and processed by image analysis software. For skin color analysis, a rectangular section of the caudal fin was examined on each fish for hue, intensity, and saturation values. These values were compared with *t*-tests.

Near the time of release, 30 fish were sacrificed from each raceway for fish health examinations. In 2001, these examinations occurred on 10 May at Kendall Creek Hatchery, on 27 April at Soos Creek Hatchery (on a subsample of fish held back from the main release), and

on 4 May at Minter Creek Hatchery. In each examination, the fish were first euthanized in MS 222 and then the external condition of the fish assessed using the Goede Index (Adams et al. 1993). Blood samples were then drawn to assess each fish's hematocrit, leukocrit, and serum protein profile. The coelomic cavity was then opened and the condition of major internal organs assessed using the Goede Index. A kidney smear was then plated on TSA agar to assay pathogen presence. Morphological and pathogen presence data were compared with 2 by 2 contingency table analysis. Blood parameters were arcsine transformed (hematocrit only) and compared with *t*-tests.

BY 2000 Experimental Rearing Period

Growth sampling and photography methods were the same as in the BY 1999 experimental rearing period, with the exception that a digital camera (Nikon D1) was used instead of the 35 mm slide film for some sampling dates. All Kendall Creek Hatchery photosampling was done using the digital camera. Other hatcheries were sampled with a mix of digital and slide technology. Slides were digitized using a Nikon LS-2000 slide film scanner.

Fish health examinations in 2002 took place on 10 May at Kendall Creek Hatchery, 12 April at Sol Duc River Hatchery, 1 May at Minter Creek Hatchery, 4 April at Soos Creek Hatchery, and 9 April at Issaquah Hatchery. All samples were taken at or immediately prior to release. Both hematocrit and leukocrit values were arcsine transformed prior to analysis. Otherwise fish health assessment was identical to 2001.

Smolt-to-Adult Survival Evaluation

BY 1999 Experimental Rearing Period

Fish were released on site at each of the facilities following standard WDFW protocols. The fish at Kendall Creek Hatchery were released on 16 May 2001 following 18 weeks of experimental rearing. On 19 April 2001, following 8 weeks of experimental rearing, fish were released from Soos Creek Hatchery. At Minter Creek Hatchery fish were volitionally released overnight on both 15 and 16 May 2001, and all remaining fish were forcefully released on 17 May 2001 after 11 weeks of experimental rearing.

BY 2000 Experimental Rearing Period

Fish releases in 2002 were done as in 2001. Fish were released from Kendall Creek Hatchery on 15 May 2002, after 41 weeks in experimental rearing. Fish were released from Sol Duc River Hatchery on 15 April 2002, after 18 weeks of experimental rearing. At Minter Creek Hatchery fish were released on 1 May 2002, after 19.5 weeks of experimental rearing. Soos Creek Hatchery released the fish on 8 April 2002, following 17 weeks of experimental rearing. Finally, Issaquah Hatchery fish were released on 15 April 2002, following 6.5 weeks in experimental habitat. All releases were forced, non-volitional releases.

Project Management

This project was collaboratively managed by NMFS and WDFW. Coded-wire tagging

and fish rearing was primarily performed by WDFW. Seminatural raceway habitat development and installation was primarily performed by NMFS. Data collection and analysis were conducted collaboratively by the two agencies.

Findings

Growth, Coloration, and Health

BY 1999 Experimental Rearing Period

All of the growth, coloration, and fish health samples for the first rearing year have now been processed and statistically analyzed. From tagging until they were placed into the experimental raceways the fish were reared in separate, but similar, vessels. The staff at each hatchery was advised to rear the fish in an identical fashion during this time period. None the less, the lag between tagging and placement into experimental raceway habitat provided an opportunity for differences to develop between the paired groups prior to experimentation. At all three facilities, sampling did not begin until at least 1 week after fish were ponded into the experimental raceways.

At all three hatcheries, some size differences already existed between the paired rearing treatments at first sampling. Fish at Kendall Creek Hatchery did not differ significantly in length one week after the initiation of experimental rearing (Fig. 1), though the control fish did weigh significantly more than their seminatural counterparts ($P = 0.021$; Fig. 2). By the second sampling period, five weeks after the beginning of the experiment, fish did not differ in either length or weight, and this similarity continued throughout the duration of rearing. At experiment initiation, fish at Soos Creek Hatchery differed significantly in length ($P = 0.025$; Fig. 3), but not in weight (Fig. 4). Size had evened out by the second sampling period, and fish remained similar in size for the duration of rearing. Minter Creek Hatchery fish differed in weight ($P = 0.029$), but not in length ($P = 0.076$) two weeks after initiation of experimental rearing (Figs. 5 and 6). These differences had disappeared by the second sampling period and did not develop again. In summary, the trend at all three rearing facilities was for the size difference between the treatments to close by time of release.

Even at first sampling, the color of the fish in the two rearing treatments differed on at least one of the three color axes (hue, saturation, and intensity). At Kendall Creek, both hue and intensity were statistically similar one week after ponding, but saturation was significantly different ($P < 0.001$; Figs. 7, 8, and 9). The difference in this variable was maintained throughout rearing. Significant differences developed in hue by the second sampling period, and differences in intensity did not appear until the third sampling period. One week prior to release, again only two of the three variables differed significantly, with intensity being no longer significant ($P = 0.053$).

At Soos Creek, saturation was also the only one of the three coloration variables to differ significantly at the first sampling period ($P = 0.005$; Figs. 10, 11, and 12). Differences in hue were not detected until the last coloration sample, but intensity differences were significant

($P = 0.008$) by the second sampling period. All three color axes were significantly different at the final sample.

At Minter Creek neither hue nor saturation differed significantly in the first coloration sample (Figs. 13 and 14), though intensity was significantly different ($P < 0.001$; Fig. 15). All three color axes were significantly different one month later. These color differences were maintained through the final coloration sample 14 May 2001.

In summary, by release, it appears measurable color differences had developed between the fish in the two rearing treatments at all three experimental facilities.

In 2001, there were no consistent or major differences in the health of the fish in the two rearing treatments. At Kendall Creek Hatchery no statistically significant differences were detected in fish condition by the Goede Index (Figs. 16 and 17), except in the amount of hematocrit in the blood. Fish from the seminatural raceway displayed higher red blood cell counts ($P = 0.005$; Fig. 17). At Soos Creek Hatchery the only variable to show significant differences from the fish condition profile was the bile ($P = 0.010$; Fig. 18). None of the blood variables were statistically different (Fig. 19). At Minter Creek Hatchery there were no detectable differences in the fish condition profile (Figs. 20 and 21).

BY 2000 Experimental Rearing Period

All the growth, coloration, and fish health samples for this second rearing year have now been processed and statistically analyzed. From tagging until they were placed into the experimental raceways the fish were reared in separate, but similar, vessels. The staff at each hatchery was advised to rear the fish in an identical fashion during this time period. None the less, the lag between tagging and placement into experimental raceway habitat provided an opportunity for differences to develop between the paired groups prior to experimentation. The time from fish distribution into the experimental raceways to first sampling varied between hatcheries.

In 2002, unlike in 2001, fish in the two treatments were similar in size at four of the five hatcheries at the beginning of sampling. Only Kendall Creek Hatchery had size differences, which was also the only hatchery where fish had been in the raceways for longer than 1 month prior to sampling initiation. Both length ($P = 0.050$; Fig. 22) and weight ($P = 0.035$; Fig. 23) were significantly different at this time. These size differences disappeared by the second sampling and were not observed again through release. Fish at both Sol Duc and Minter Creek hatcheries remained similar in size throughout the duration of the study (Figs. 24, 25, 26, and 27). Fish size did not differ significantly at Soos Creek Hatchery at either the first or second sample (Figs. 28 and 29). Both length ($P = 0.005$) and weight ($P = 0.004$) were significantly different in March, but these differences were removed by release. Issaquah Hatchery coho salmon were not significantly different in size at the beginning of experimental rearing (Figs. 30 and 31), but both length ($P = 0.007$) and weight ($P = 0.011$) were significantly different at the final sample.

At Kendall Creek Hatchery at the first sampling the coloration of fish in the two rearing

treatments differed significantly on two of the three color axes (Figs. 32, 33, and 34). Hue and saturation were significantly different ($P < 0.001$ for both), but intensity was not ($P = 0.368$). This trend was repeated one month later, and reversed the following month. At each of the last two samples, including 1 week prior to release, all three color axes differed significantly.

Sol Duc Hatchery coloration was significantly different for all three color axes at first sampling (Figs. 35, 36, and 37). One month later, hue was not significantly different ($P = 0.464$), but saturation ($P < 0.001$) and intensity ($P < 0.001$) still differed. By the third month, there were no longer any coloration differences between treatments, and likewise for the fourth sample. At the time of release, hue ($P = 0.002$) and saturation ($P < 0.001$) again differed significantly, but intensity was not significantly different ($P = 0.088$).

On the day after fish were placed into seminatural rearing habitat at Minter Creek Hatchery, saturation differed significantly ($P < 0.001$; Fig. 38) between treatments, but not hue or intensity (Figs. 39 and 40). One month later, significant differences in hue had developed ($P = 0.004$), saturation differences remained, but intensity differences had not developed. Inversely, at the third sampling date, neither hue nor saturation differed significantly, but intensity did ($P < 0.001$). By the fourth sampling date, hue and saturation differences returned, with intensity again being similar. None of the three color axes differed significantly at the fifth sampling. On the day of release, hue was statistically similar, but saturation ($P < 0.001$) and intensity ($P = 0.018$) were significantly different.

At Soos Creek Hatchery, hue differences ($P < 0.001$; Fig. 41) had developed by first sampling, approximately three weeks after initiation of experimental rearing, while saturation and intensity were similar (Figs. 42 and 43). Of the three color axes, only intensity differed significantly ($P = 0.005$) at the time of the second sample. Both hue ($P < 0.001$) and intensity ($P = 0.001$) were significantly different by the third sample. All three color axes differed significantly by the final sample.

Coho salmon at Issaquah Hatchery did not differ in coloration at the first sampling date, less than 1 week after placement into experimental rearing habitat (Figs. 44, 45, and 46). At the time of final sampling, however, all three color axes differed significantly.

In 2002, there were no consistent or major differences in the health of the fish in the two rearing treatments. At Kendall Creek Hatchery, no statistically significant differences were detected in fish condition by the Goede Index (Figs. 47 and 48). There were also no statistically significant differences in fish condition or blood variables at Sol Duc River Hatchery (Figs. 49 and 50), nor at Minter Creek Hatchery (Figs. 51 and 52). At Soos Creek Hatchery, no statistically significant differences were detected in fish condition by the Goede Index (Figs. 53 and 54), except in the amount of hematocrit in the blood. Fish from the seminatural raceway displayed lower red blood cell counts ($P < 0.001$; Fig. 54). Issaquah Hatchery coho salmon displayed no differences in fish condition (Fig. 55), except for elevated plasma protein in the blood of seminaturally-reared coho salmon ($P = 0.009$; Fig. 56). Hematocrit was not significantly different (Fig. 56).

Smolt-to-Adult Survival Evaluation

Data on returns of coho salmon released in 2001 will not be available until 2004. Return data of coho salmon released in 2002 should be available for analysis beginning in 2005.

General Observations

In both rearing years, the Kendall Creek Hatchery, Soos Creek Hatchery, and Minter Creek Hatchery pavers were neither fouled by algae nor buried by sand. However, at Sol Duc Hatchery the pavers in both the control and experimental Burrow's ponds were covered by a lush brown algae mat and no substrate color differences could be distinguished over most of the pond bottoms. At Issaquah Creek Hatchery, both raceways were covered with a thick layer of fine sand for at least half their length. Inspection with an underwater video camera suggested there were no differences in substrate color for at least half of these pond lengths. Differences in substrate rugosity were maintained at both these facilities as well as at the other three used two years in a row.

Discussion

The structural components used for the seminatural raceway habitat have been refined to be compatible with daily salmon culture activities. The concrete pavers are easily installed and long-lasting, at a cost of \$6.50/ft². This cost may be further reduced by switching from custom handmade to production line fabrication. Their rugosity helps settle solids (waste and sediment) out of the water column, while still providing a mottled background. The hydraulic lifts used on the hinged covers at all but Minter Creek Hatchery made for easy operation by hatchery staff. The exception to this was the occurrence of broken welds on the frames, requiring repairs by off-site staff. By reinforcing the welds on the hinged frames, this problem can most likely be avoided in the future. Unfortunately, the cost of these frames is high (\$10.00/ft²) with little chance of being lowered without losing the hinge and self-lifting design. The problem with snow loads ripping the camouflage covers at Minter Creek Hatchery was resolved this year by suspending the camouflage under the frame, rather than draping it over the frame. The fir tree instream structure was almost problem-free. It required no maintenance and hatchery personnel could vacuum the raceways with it present. It now appears that we have developed a seminatural raceway habitat that can be successfully operated by fish culturists at production hatcheries, as all fish culture and raceway maintenance in this study was conducted by hatchery personnel, rather than research scientists.

The growth profiles of the fish in this study are different than that observed in past seminatural raceway habitat studies. In three of the eight cases, seminaturally-reared fish began smaller in size than conventionally-reared fish, and outgrew their conventionally-reared counterparts, ending either similarly in size or even larger than the conventionally-reared fish. In four of the eight cases, fish from the two treatments began similar in size, and ended with seminaturally-reared fish slightly larger than conventionally-reared fish (though in only one instance was the difference statistically significant). Finally, only in the 2001 Soos Creek replicate did the seminaturally-reared fish fail to outgrow the conventionally-reared fish in culture. In most of our previous chinook salmon studies, the trend was for conventionally-reared

fish to grow faster than seminaturally-reared fish (Maynard et al. 1996a,b,d). With that species, the different growth profile was attributed to their reluctance to feed on food that has fallen into spaces between the gravel in the seminatural raceways. However, hatchery coho salmon are not known to be reluctant to pick food up off the bottom. Therefore, with coho salmon, we can not advance any logical explanation for this potential growth difference between the treatments at this point in time.

As expected, color differences developed between the two rearing treatments at each facility before release. This is similar to the observations made on chinook salmon grown in seminatural raceway habitat in studies conducted at Big Beef Creek, Bingham Creek, and Forks Creek (Maynard et al. 1996b,d; Maynard et al. 2001). It is assumed these skin color differences, which visually match stream and river substrate better than fish reared in concrete colored raceways, enable seminaturally-reared fish to better blend into the stream and river background. This enhanced camouflage coloration should result in the fish being less visible to predators and produce increases in postrelease survival.

The time series development of hue, saturation, and intensity differences between the two rearing treatments did not develop as clear a pattern as observed in a previous study (Maynard et al. 2001). This may be due to the fact that previous studies included frequently removing algae and sediment that covered raceway bottoms and sides. This extra effort is not made in the current study. Thus, the between sampling period shifts in skin coloration observed at each facility may be a response to seasonal changes in floral growth and sediment buildup on rearing pond bottoms rather than a response to NATURES substrate.

In FY 2004, color analysis will include raceway floor color measurement to determine if skin coloration is developing to match the background over which the fish are reared. It is presumed that the hue, saturation, and intensity of the color of control fish will match their plain concrete raceway bottom, while that of seminaturally-reared fish will match that of the pavers and associated flora over which they are reared.

As in past experiments, the health of the fish in seminatural and conventional raceway habitat appeared to be similar. The differences that did occur were not consistent across all rearing facilities, suggesting major treatment differences do not exist.

The preliminary study findings indicate that seminatural raceway habitat can be operated at production scale facilities and produce apparent beneficial biological rearing effects in coho salmon similar to those that seem to improve the instream postrelease survival of chinook salmon. Fishery managers can use this increased postrelease survival to improve hatchery efficiency, increase harvest, speed the rebuilding of self-sustaining natural runs through supplementation, or simply reduce the ecological impact of hatchery fish by lowering release numbers while maintaining recruitment.

Two more years of rearing and release are required to complete the original experimental design. Beginning in 2003, it may be possible to monitor instream survival from Issaquah Hatchery using the PIT-tag detector system present on the Lake Washington Ship Canal. This would only require additional tagging work and would provide juvenile migration data until the adult return data was complete.

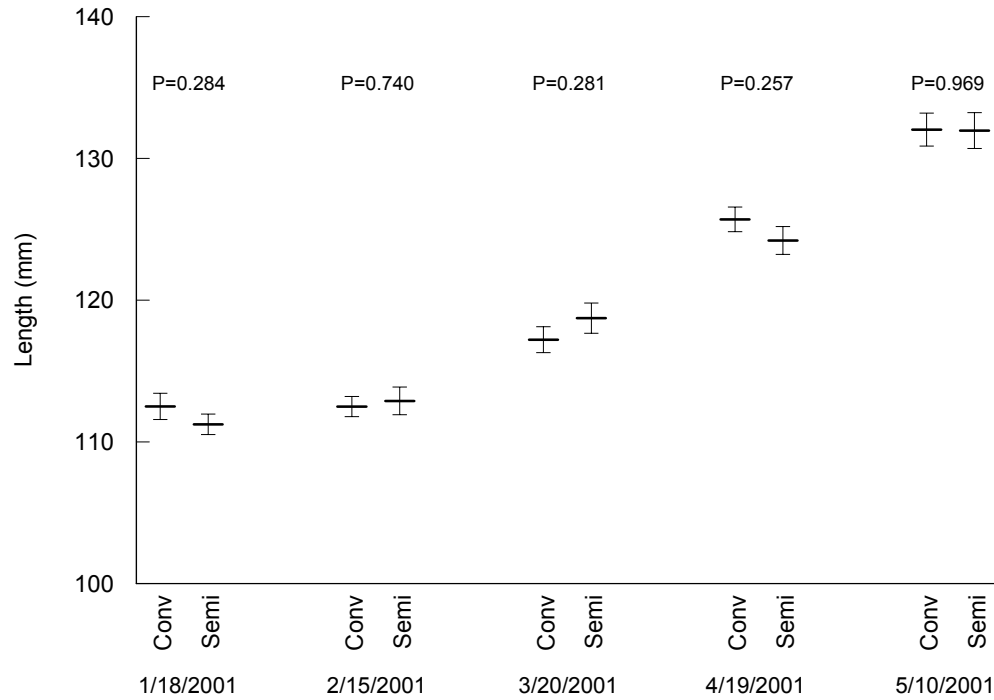


Figure 1. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/10/2001). P values are based on *t*-tests.

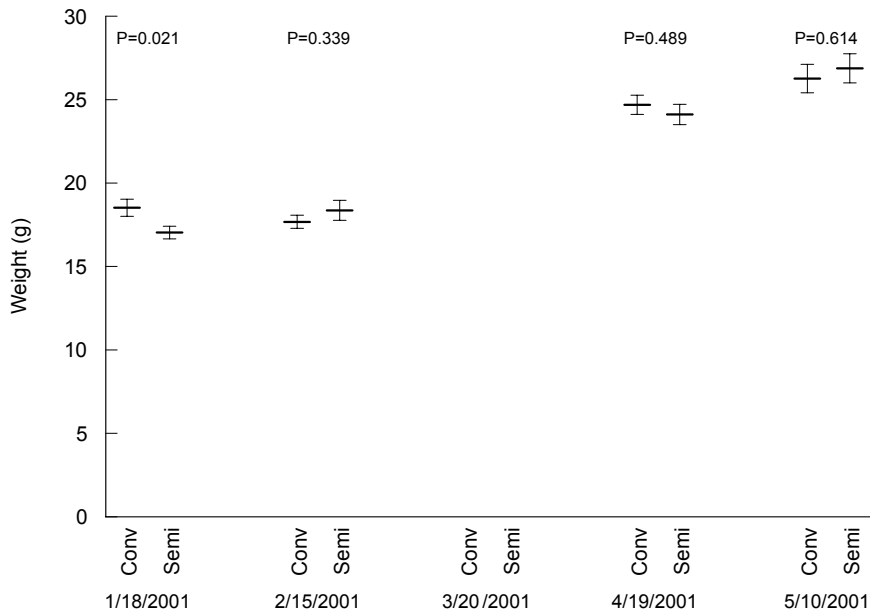


Figure 2. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/10/2001). P values are based on *t*-tests.

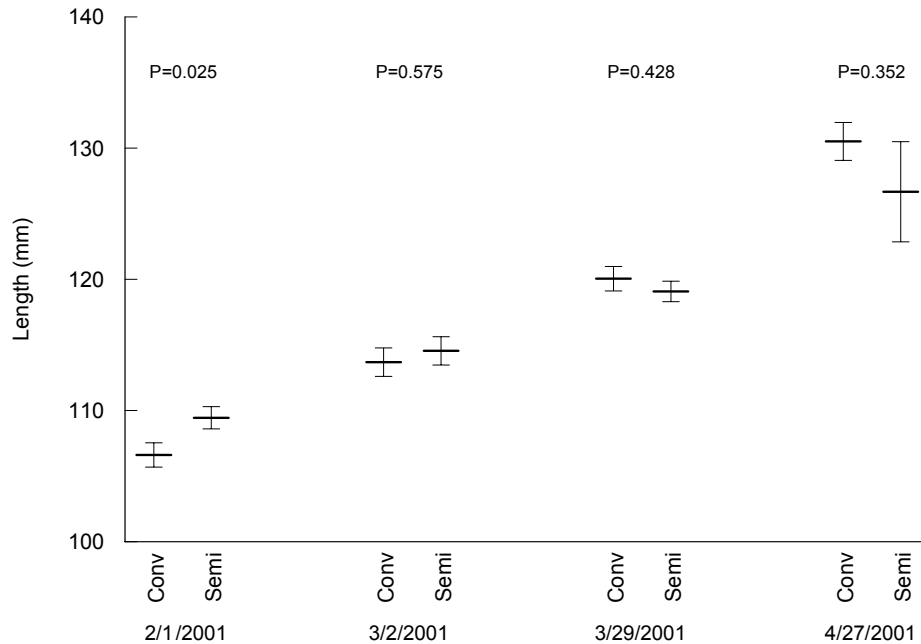


Figure 3. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 4/27/2001). P values are based on *t*-tests.

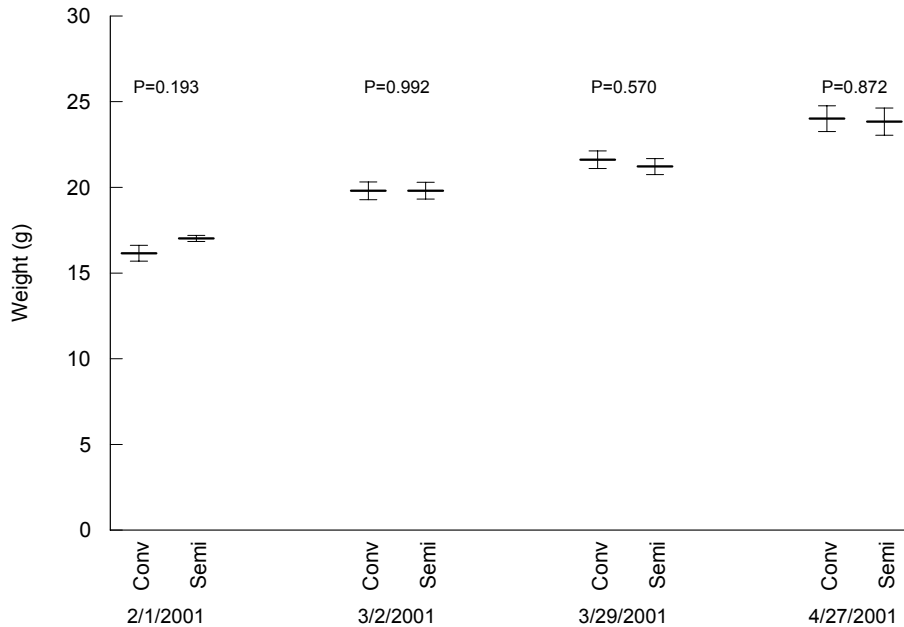


Figure 4. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 4/27/2001). P values are based on *t*-tests.

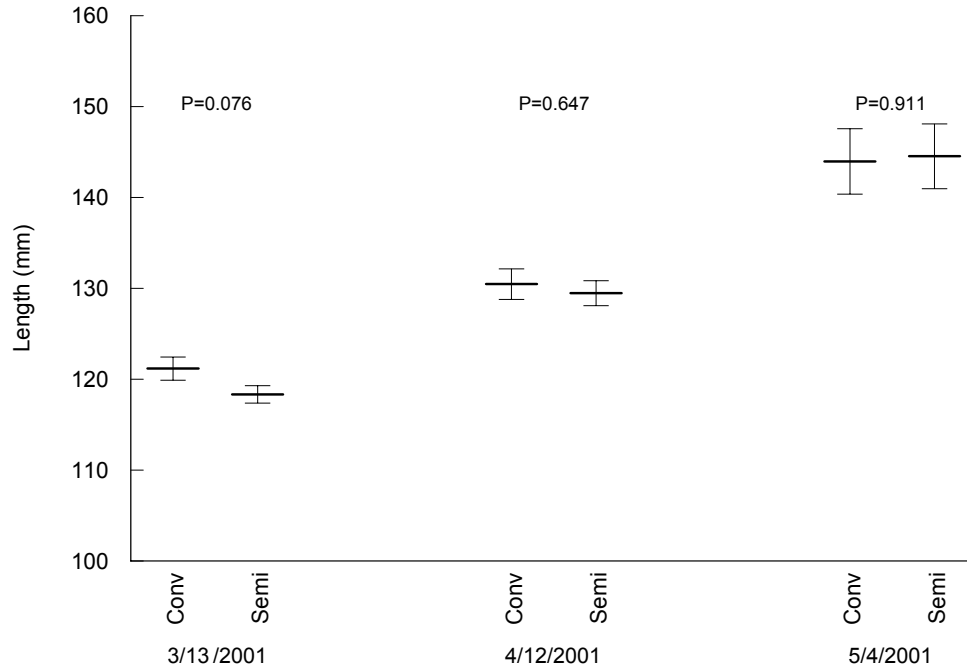


Figure 5. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/4/2001). P values are based on *t*-tests.

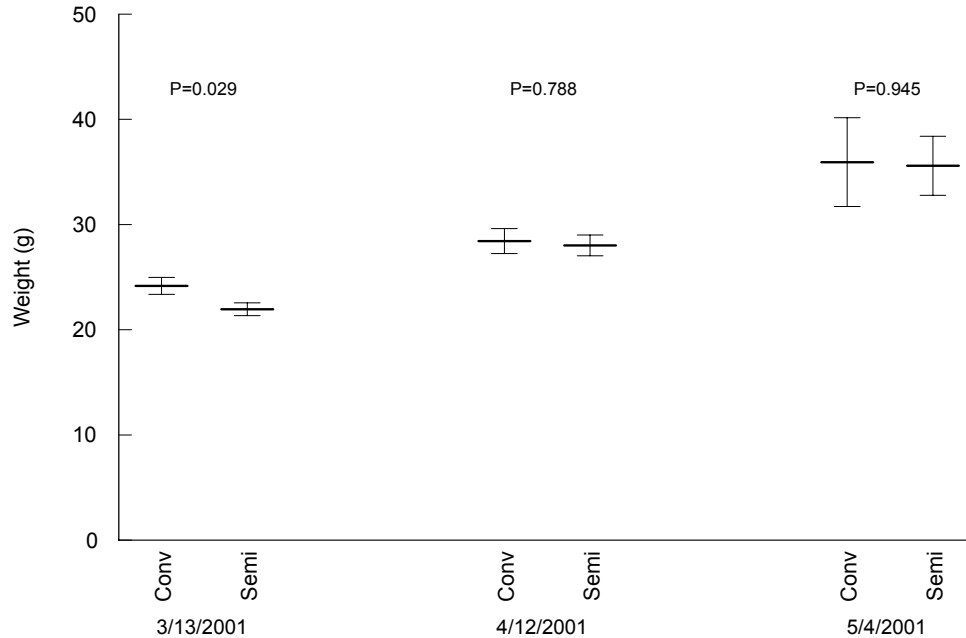


Figure 6. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 100 per treatment, except N = 30 per treatment on 5/4/2001). P values are based on *t*-tests.

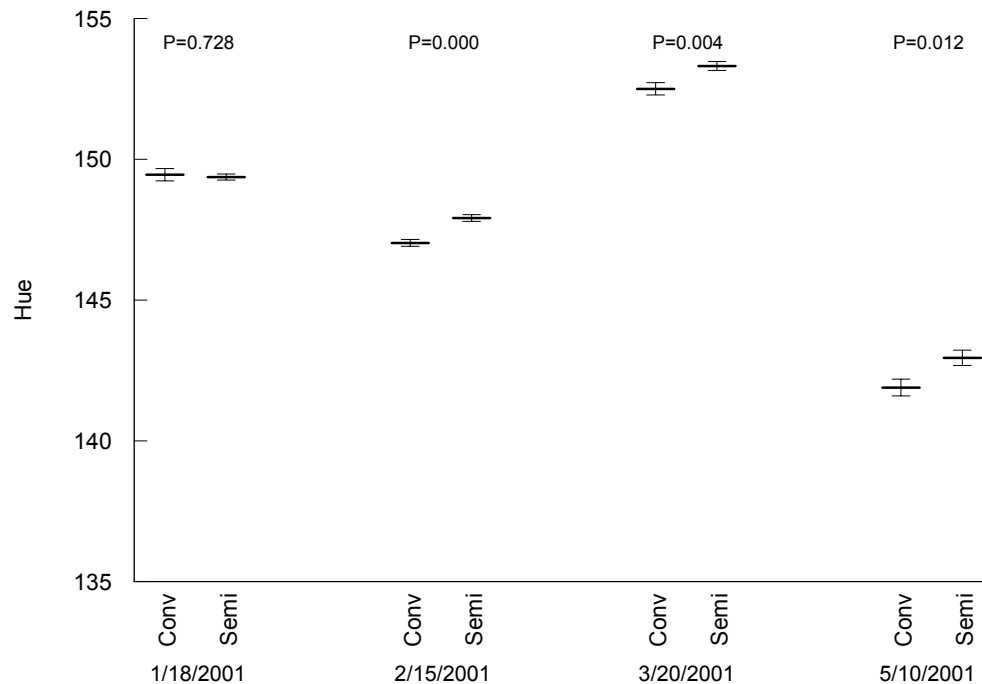


Figure 7. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

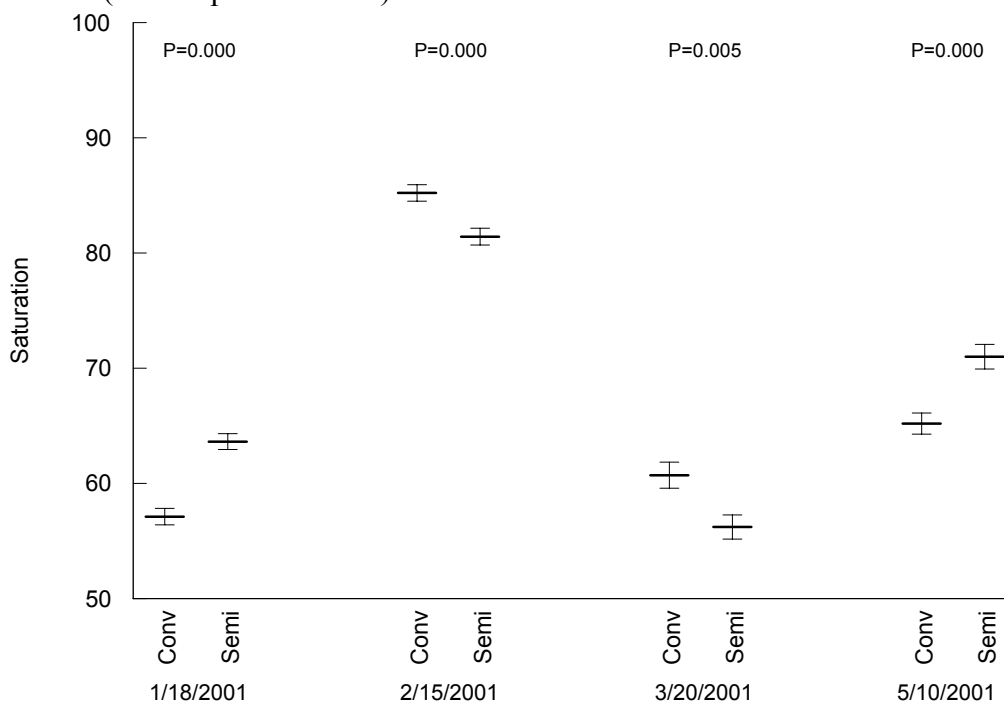


Figure 8. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

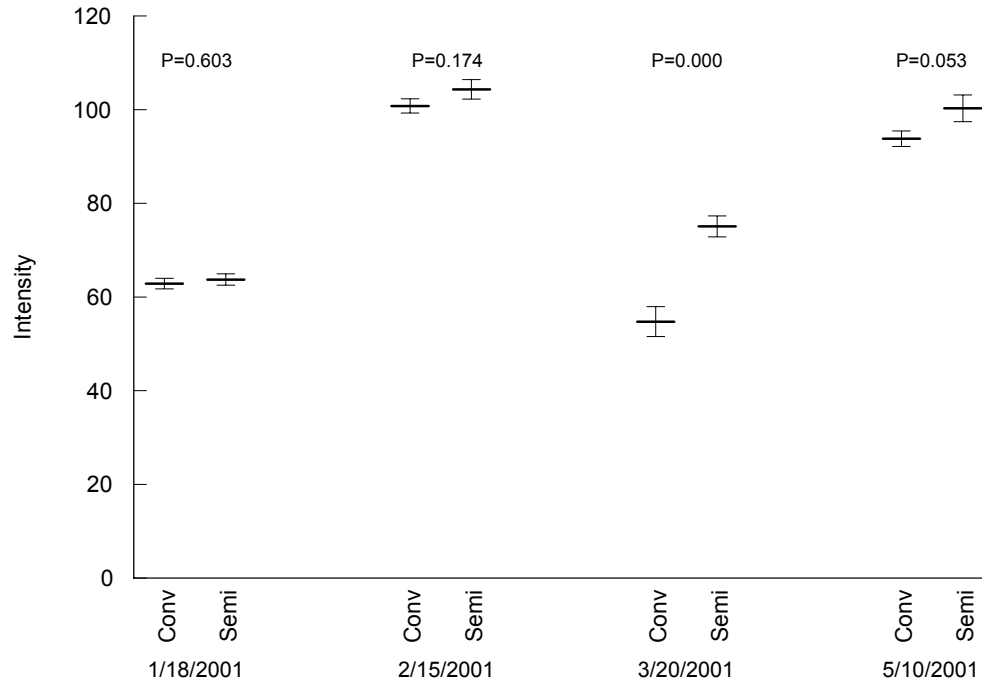


Figure 9. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

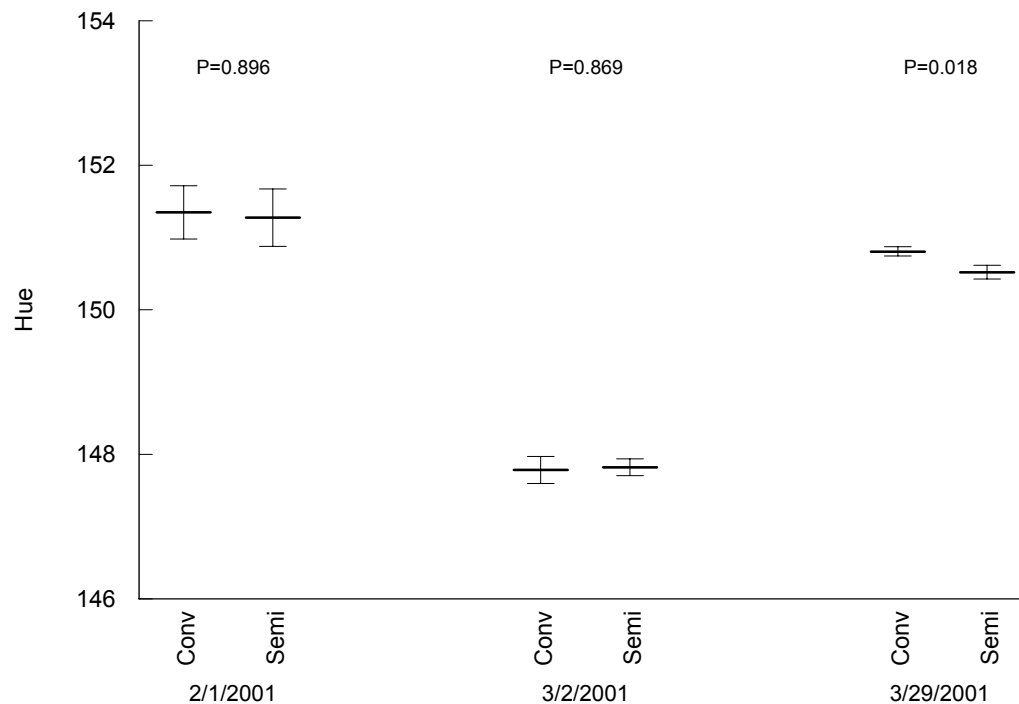


Figure 10. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

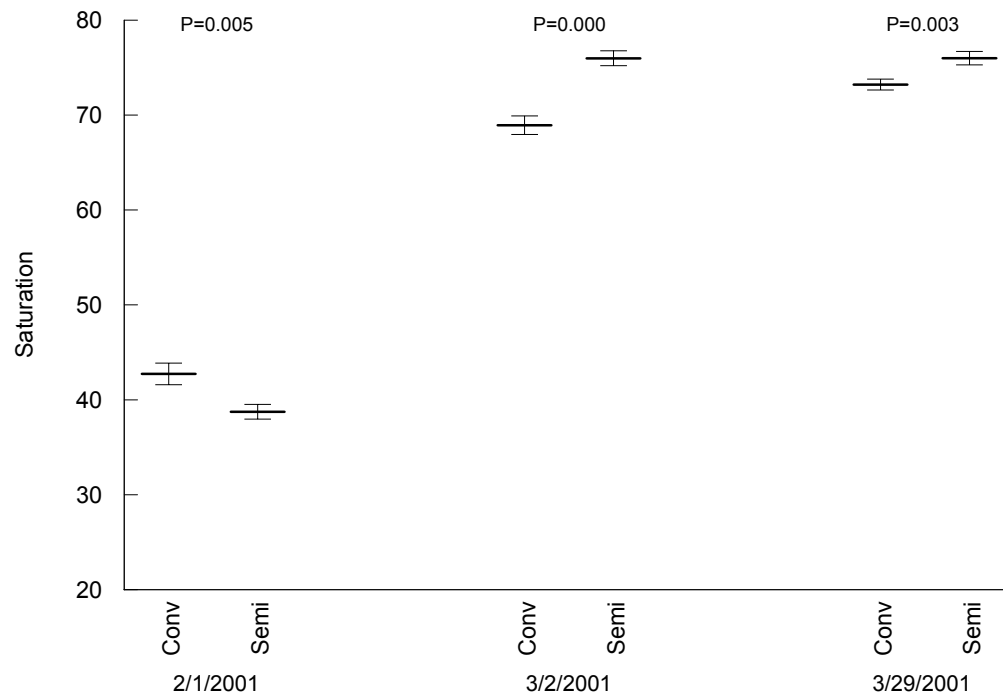


Figure 11. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

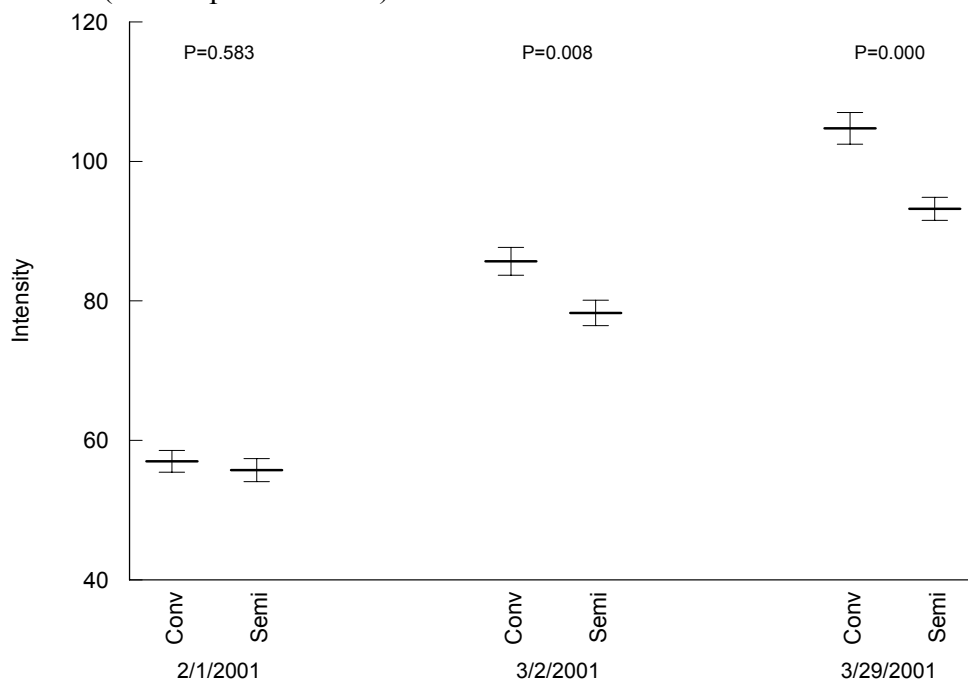


Figure 12. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

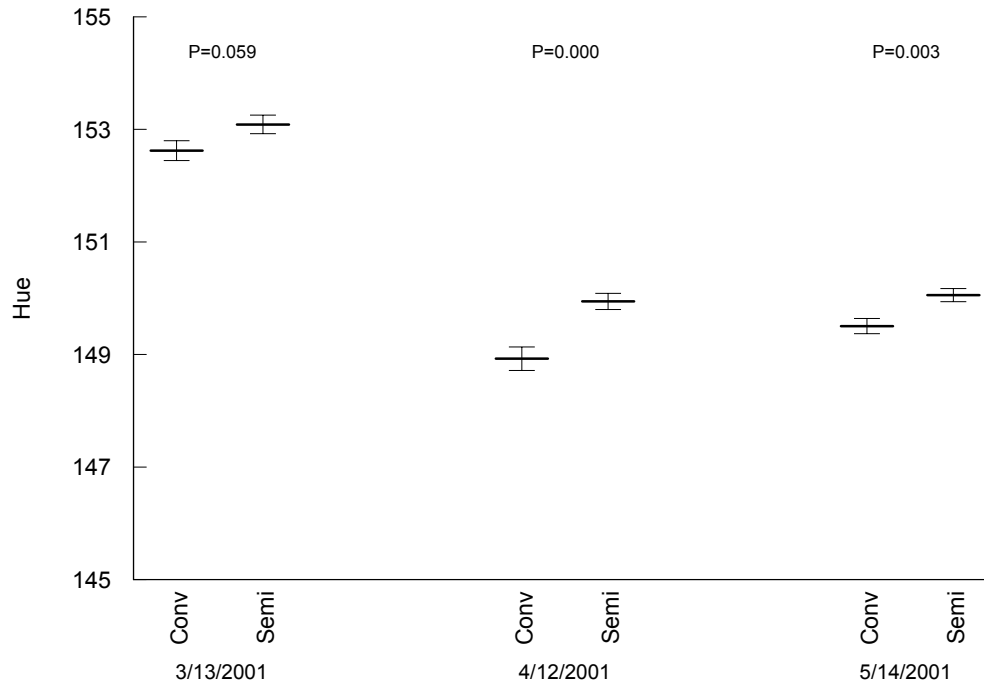


Figure 13. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

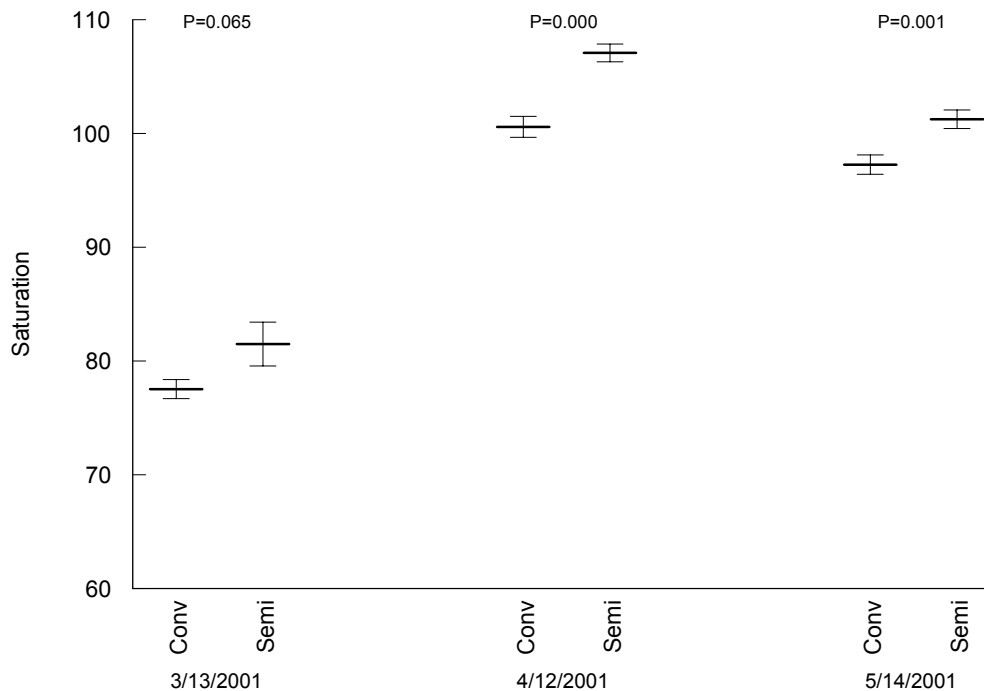


Figure 14. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

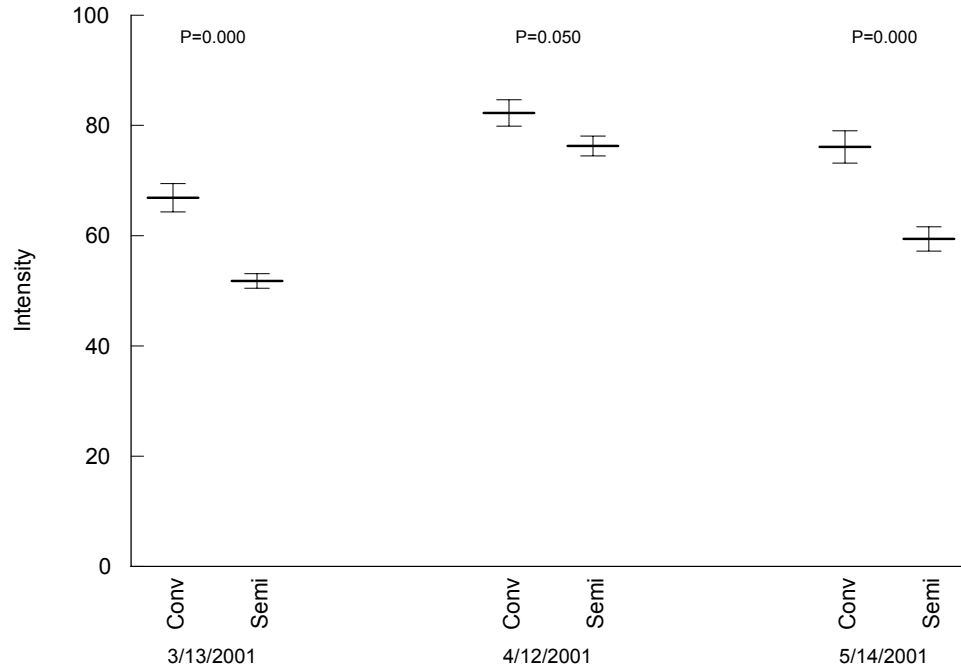


Figure 15. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2001 (N = 30 per treatment). P values are based on *t*-tests.

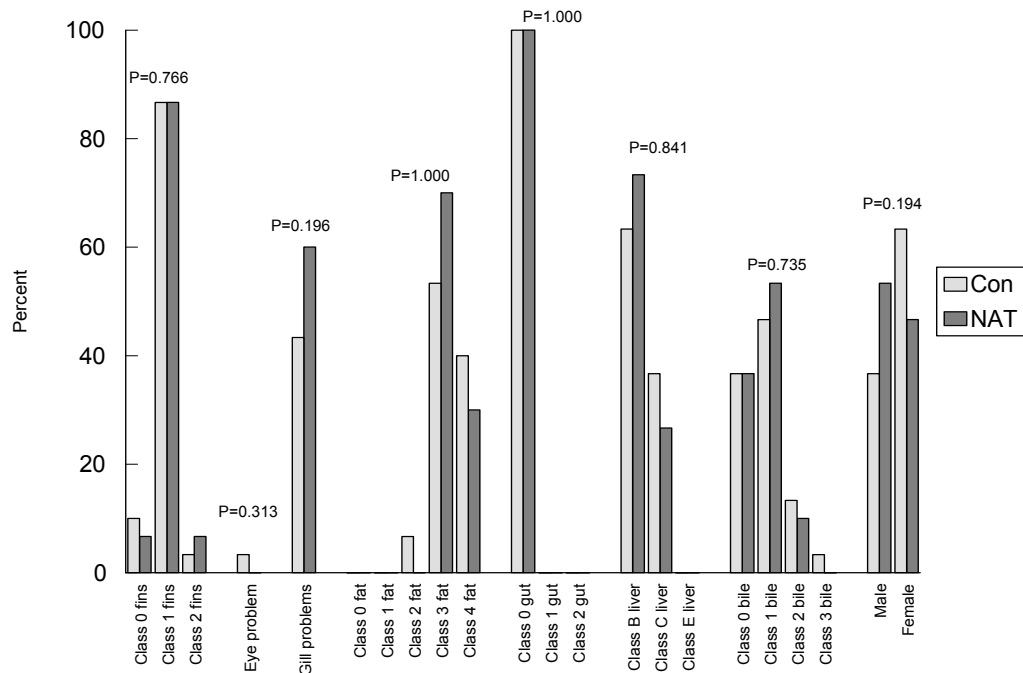


Figure 16. Percentage of coho salmon in different Goede Index classes in the 10 May 2001 Kendall Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

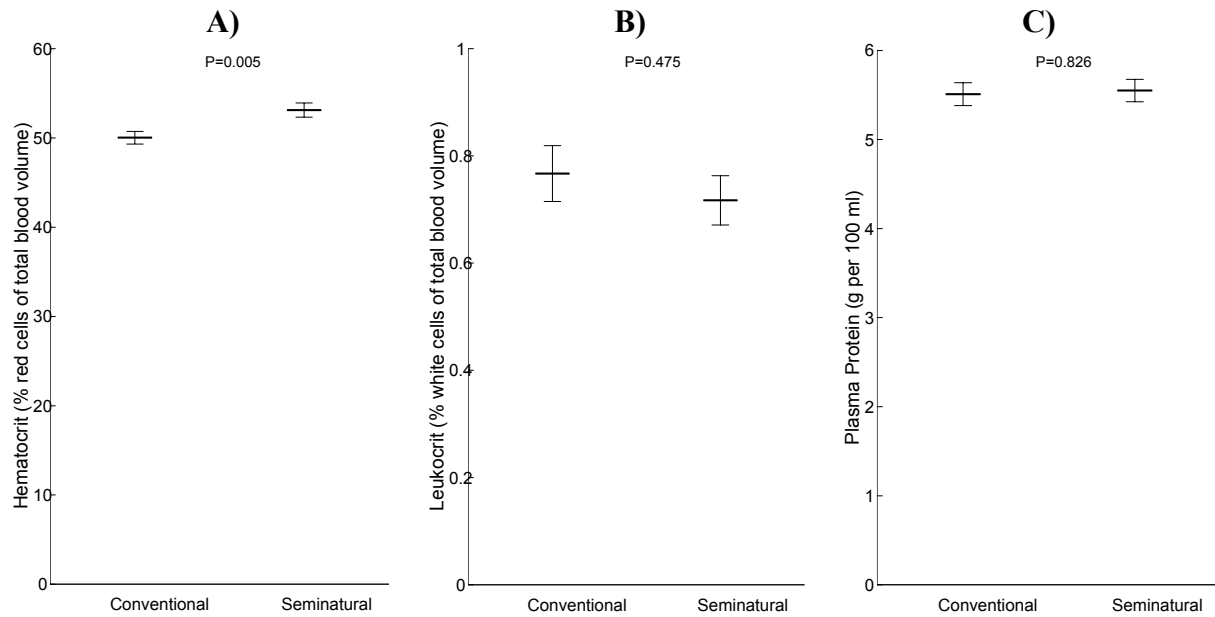


Figure 17. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Kendall Creek Hatchery sampled on 10 May 2001. A) hematocrit (N = 30 per treatment) P value based on *t*-tests of arcsine transformed data; B) leukocrit (N = 30 per treatment) P value based on *t*-tests; and C) plasma protein (N = 29 conventional and 28 seminatural) P value based on *t*-tests.

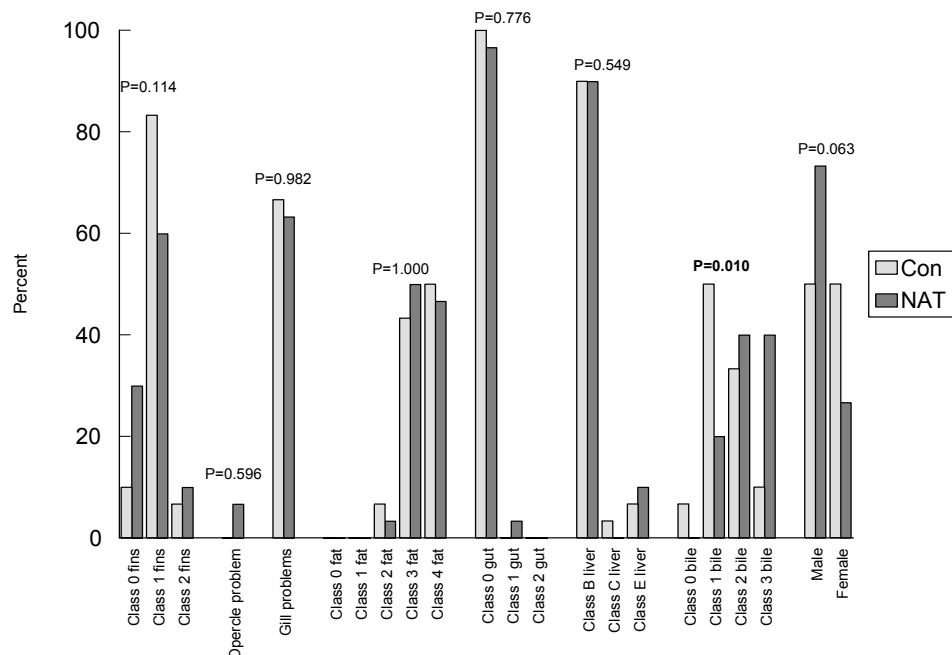


Figure 18. Percentage of coho salmon in different Goede Index classes in the 27 April 2001 Soos Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

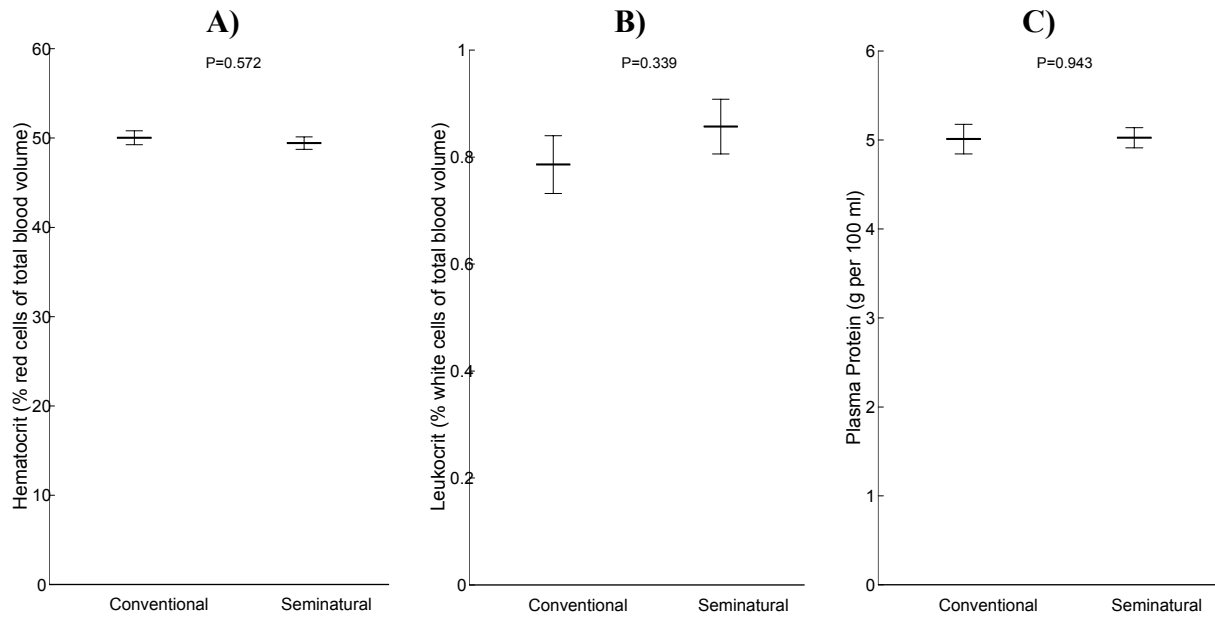


Figure 19. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Soos Creek Hatchery sampled on 27 April 2001. A) hematocrit (N = 30 per treatment) P value based on *t*-tests of arcsine transformed data; B) leukocrit (N = 28 per treatment) P value based on *t*-tests; and C) plasma protein (N = 28 per treatment) P value based on *t*-tests.

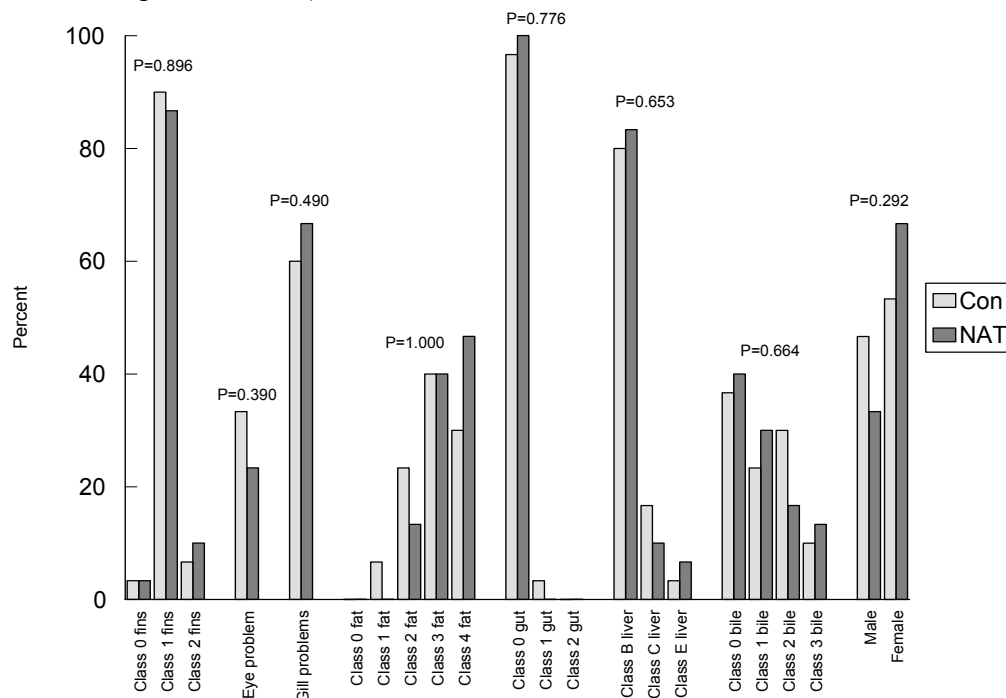


Figure 20. Percentage of coho salmon in different Goede Index classes in the 4 May 2001 Minter Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

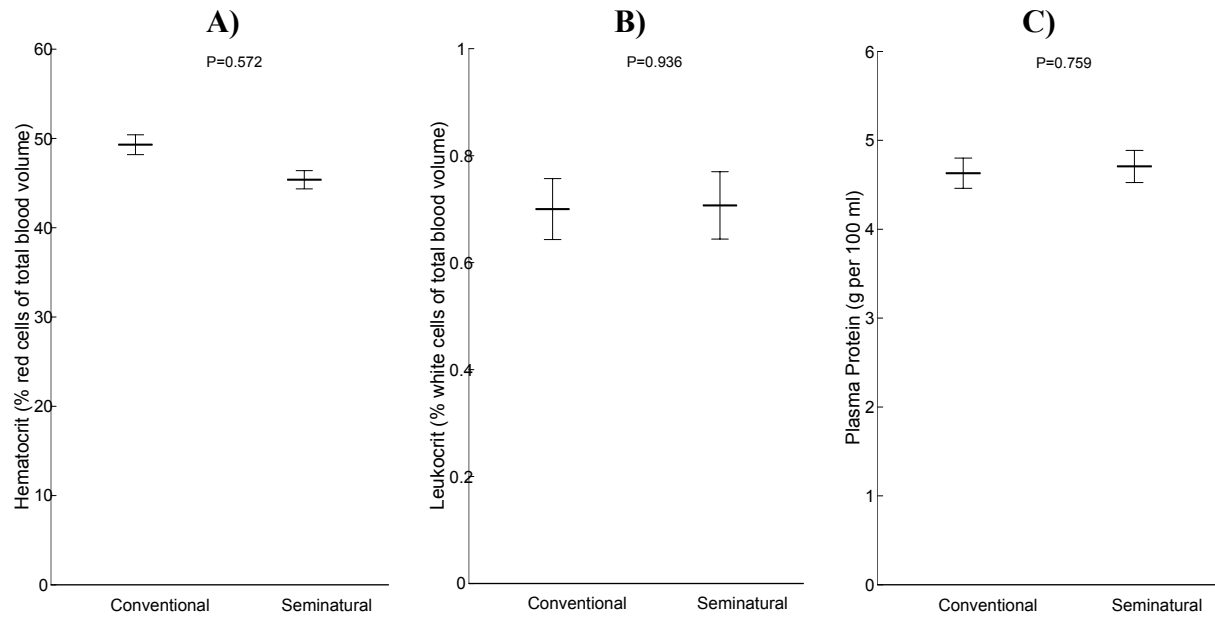


Figure 21. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Minter Creek Hatchery sampled on 4 May 2001. A) hematocrit (N = 30 conventional and 29 seminatural) P value based on *t*-tests of arcsine transformed data; B) leukocrit (N = 30 conventional and 29 seminatural) P value based on *t*-tests; and C) plasma protein (N = conventional and 30 seminatural) P value based on *t*-tests.

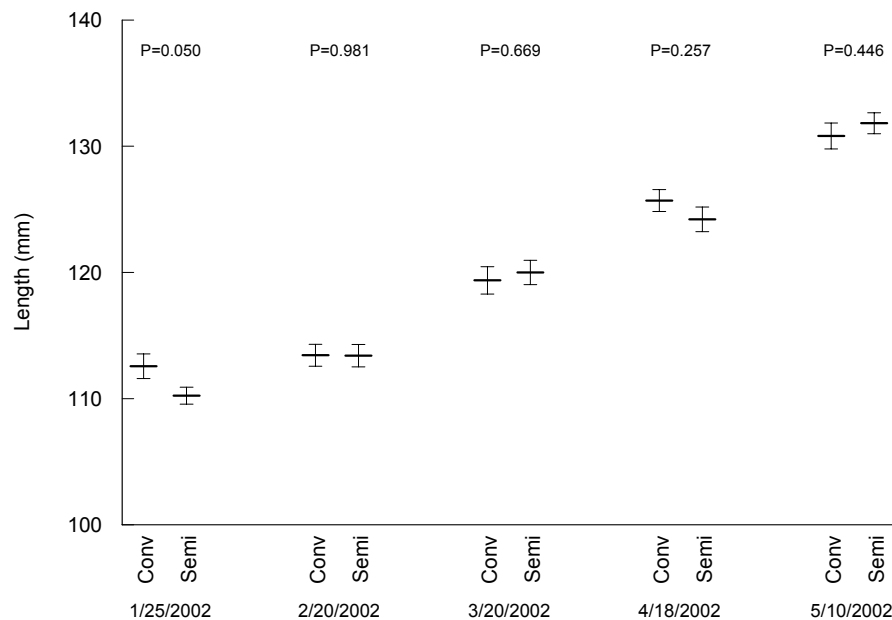


Figure 22. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

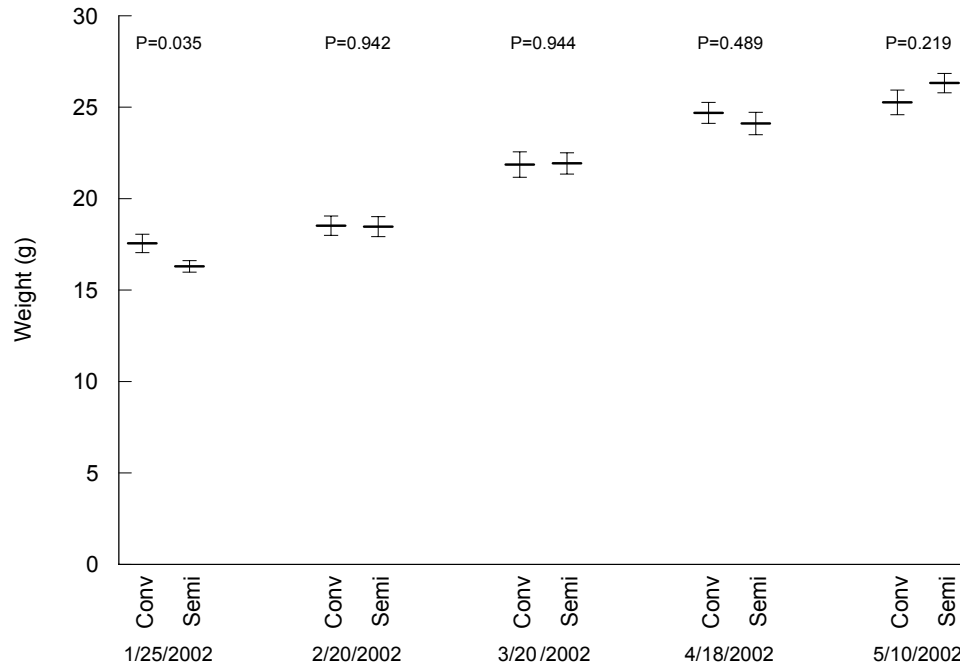


Figure 23. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

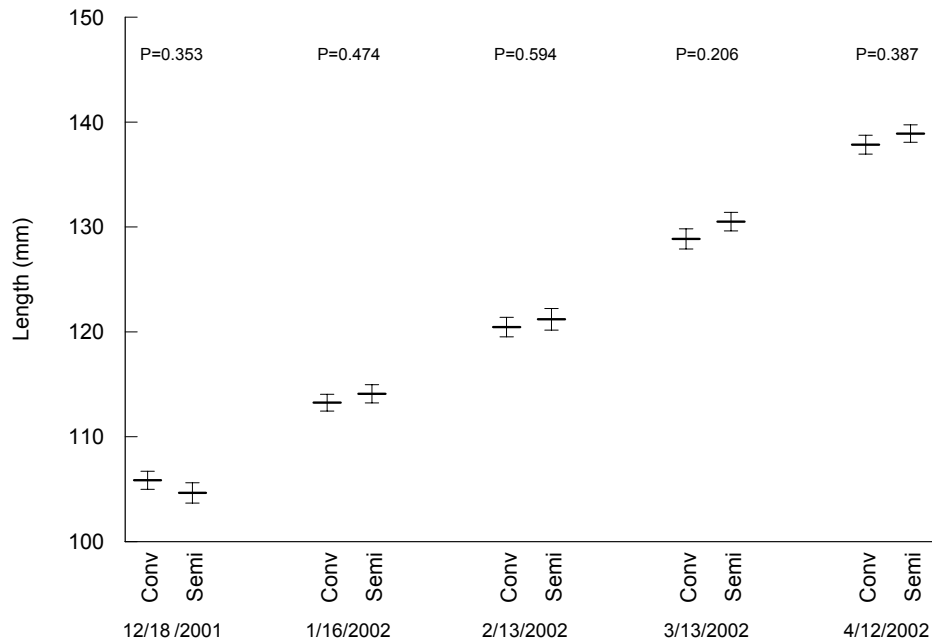


Figure 24. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

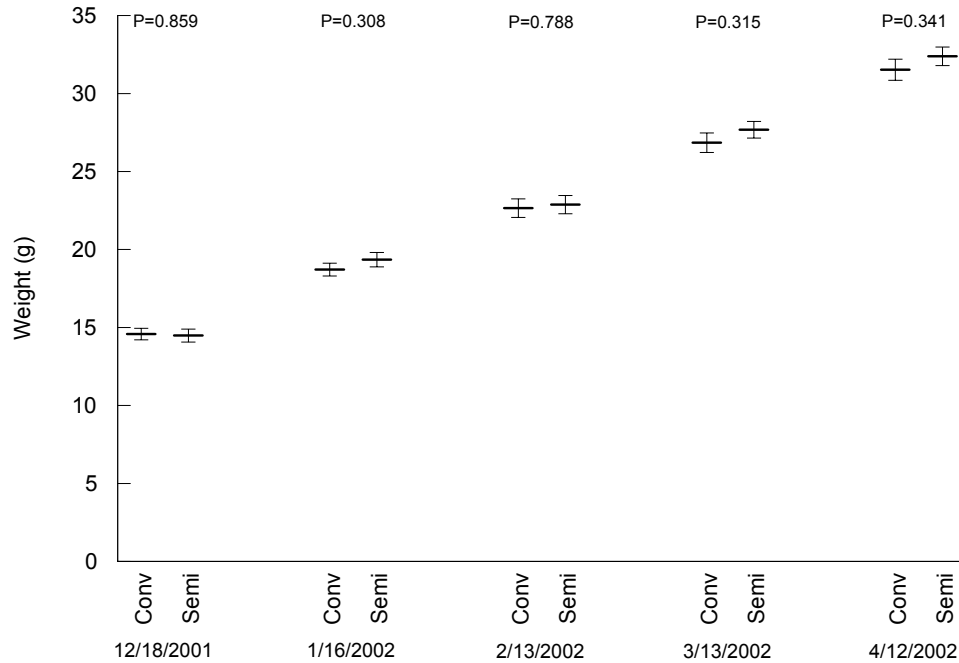


Figure 25. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

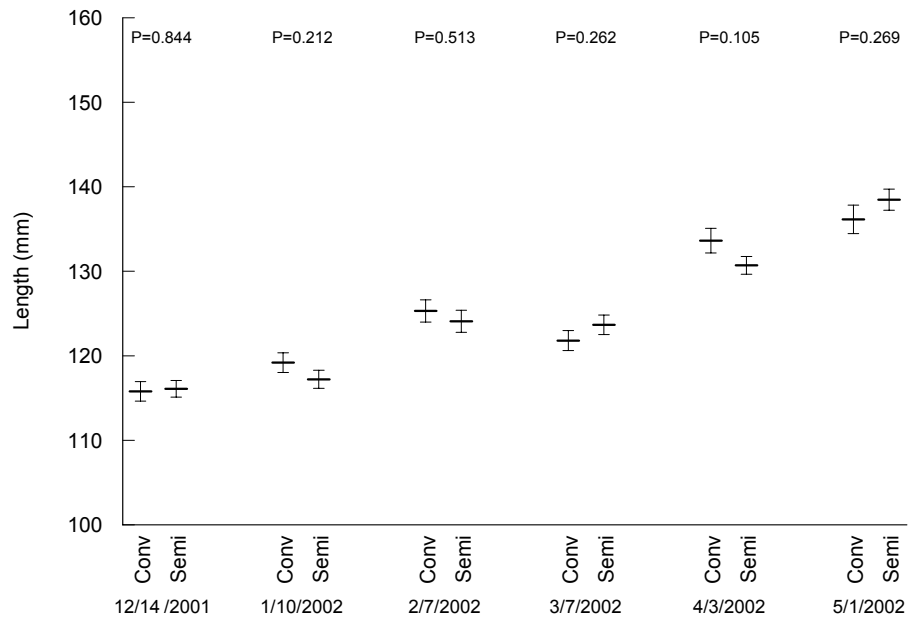


Figure 26. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

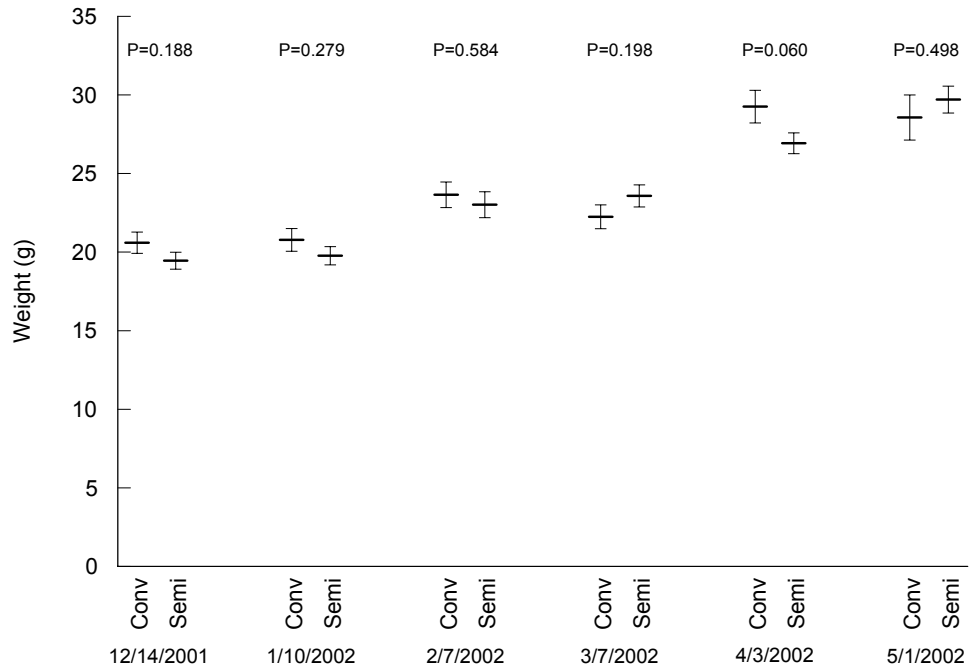


Figure 27. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

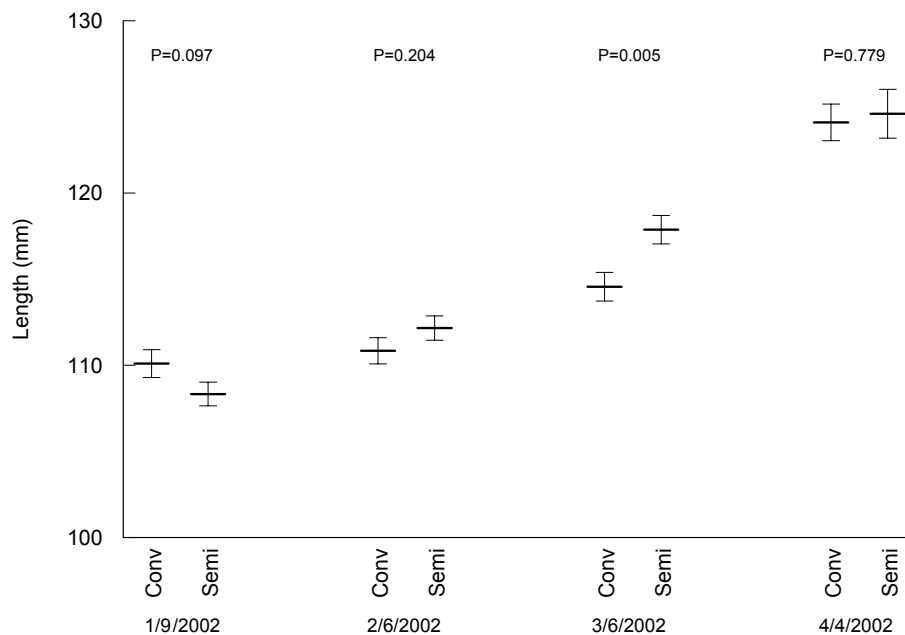


Figure 28. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 100 per treatment, except N = 30 per treatment on 4/4/2002). P values are based on *t*-tests.

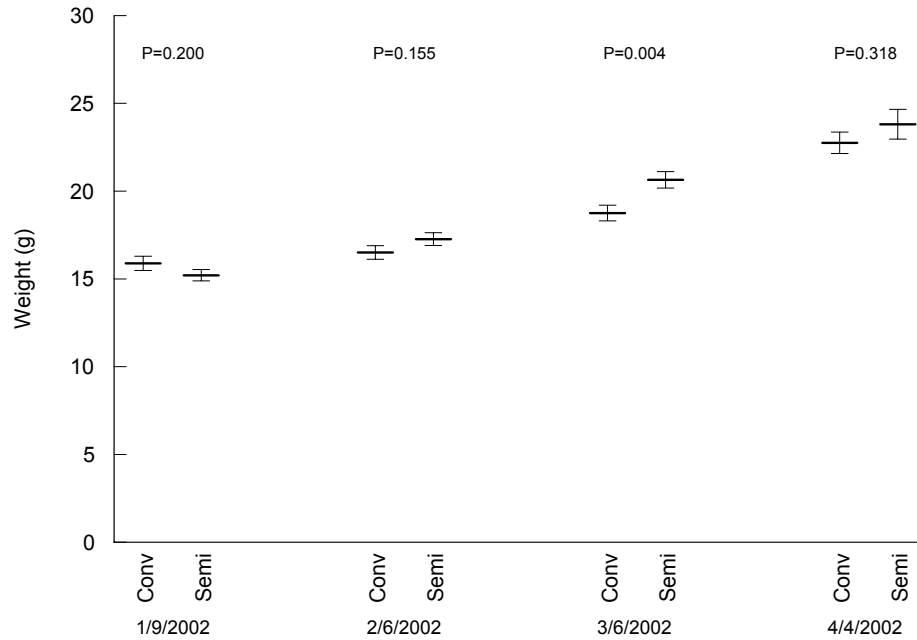


Figure 29. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 100 per treatment, except N = 30 per treatment on 4/4/2002). P values are based on *t*-tests.

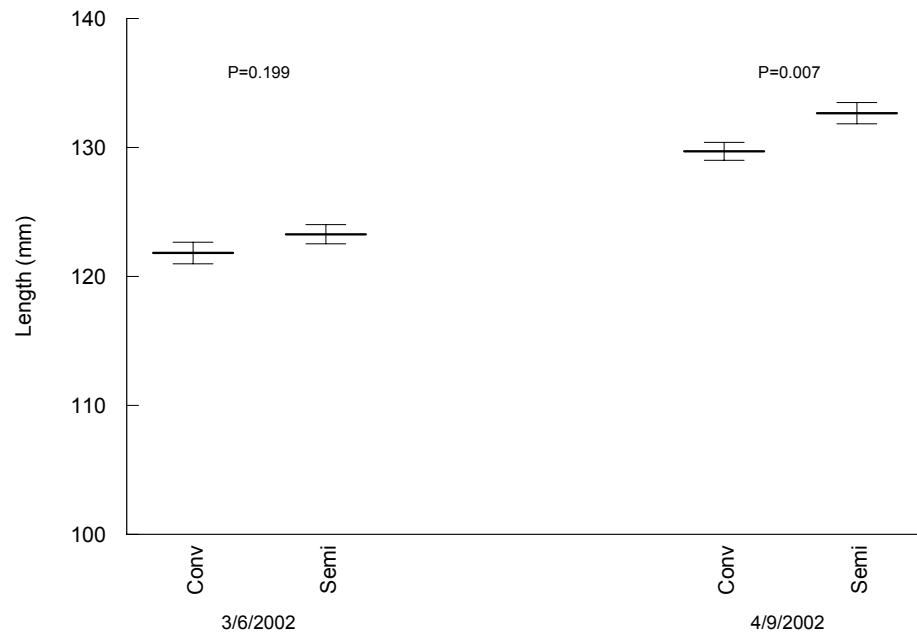


Figure 30. Mean length (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

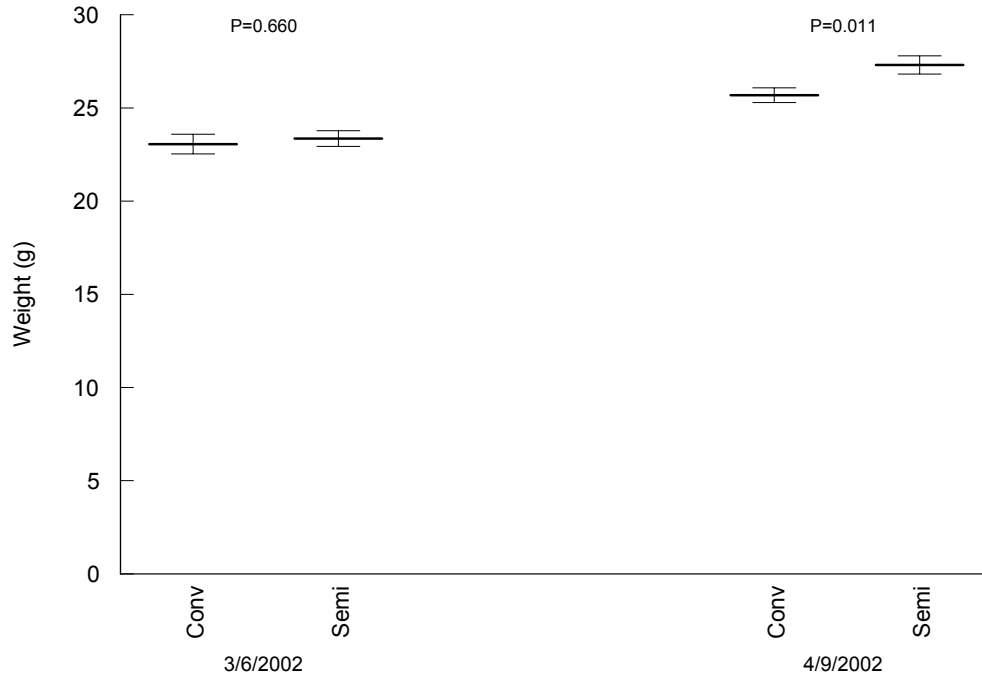


Figure 31. Mean weight (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 100 per treatment). P values are based on *t*-tests.

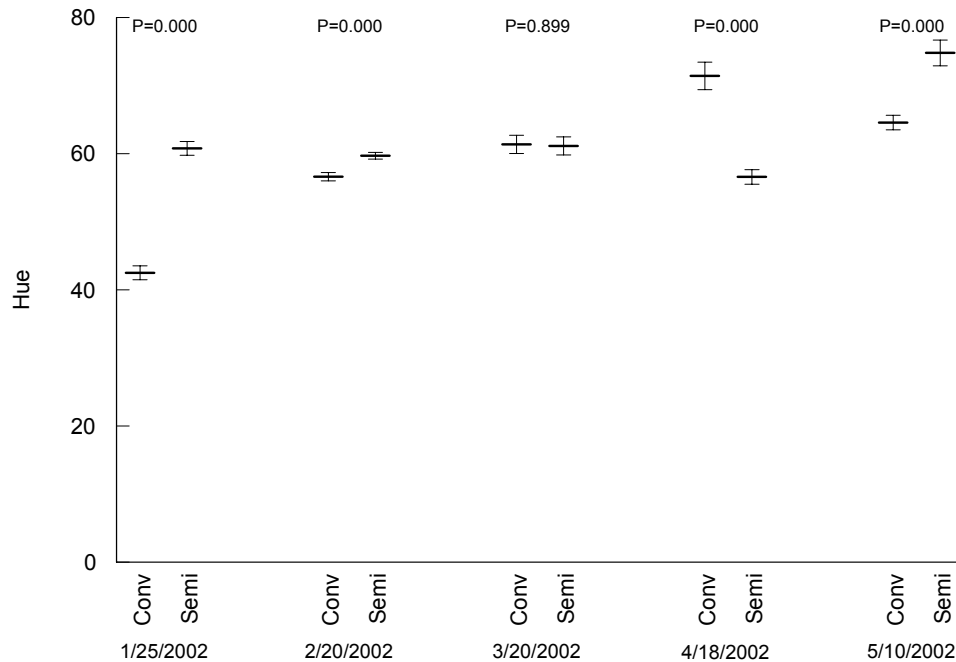


Figure 32. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

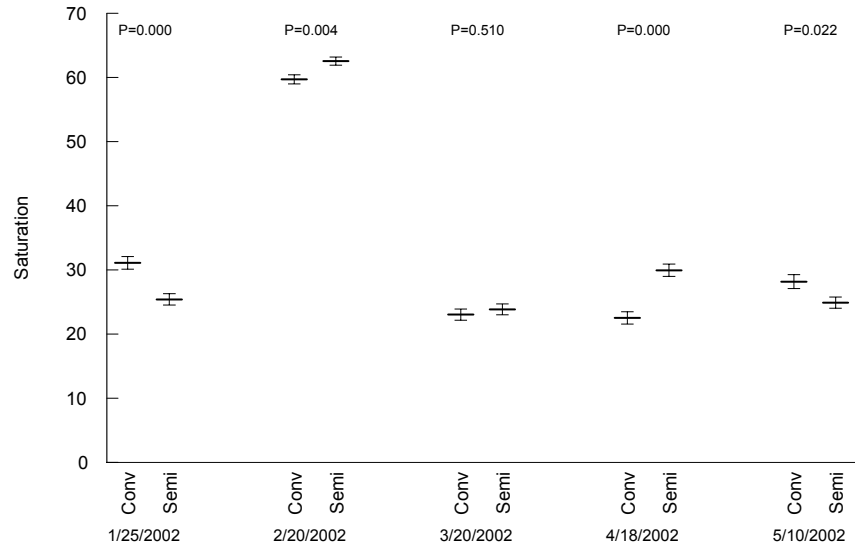


Figure 33. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

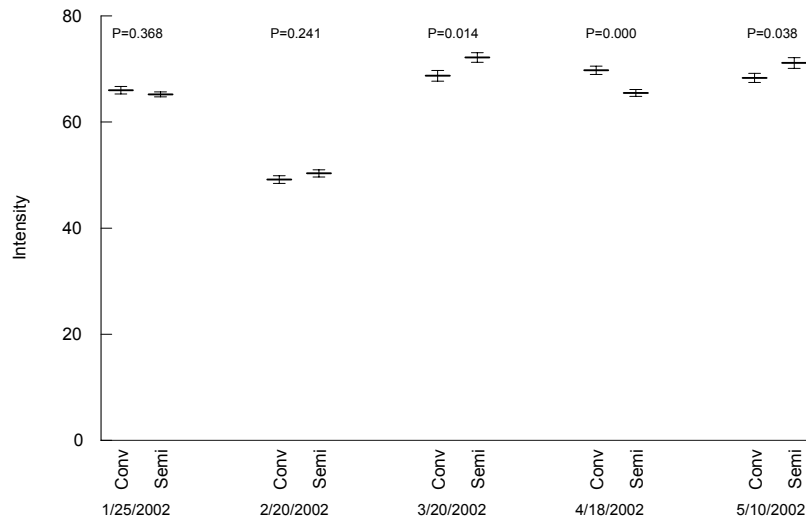


Figure 34. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Kendall Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

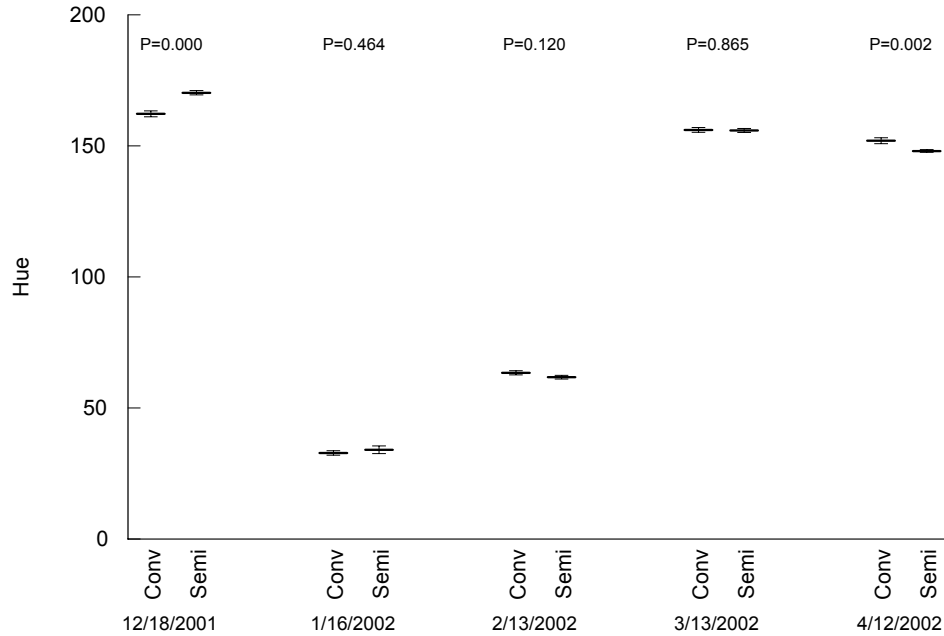


Figure 35. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

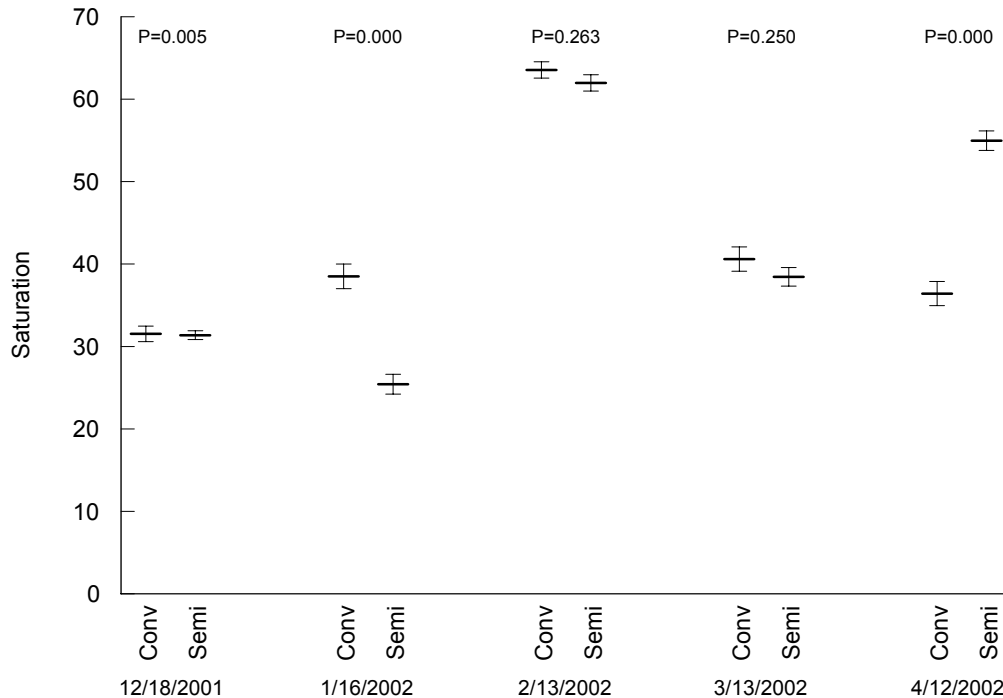


Figure 36. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

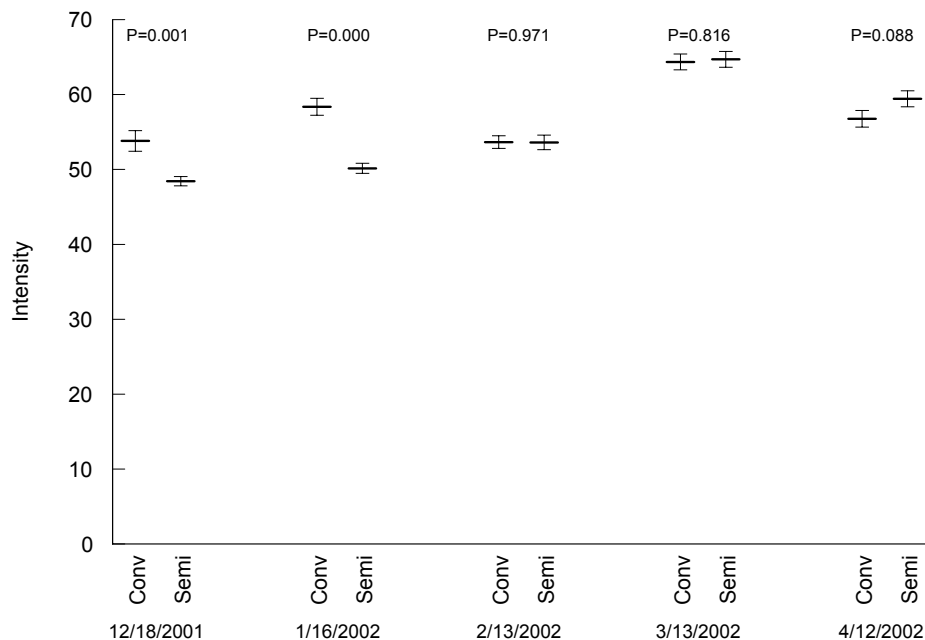


Figure 37. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Sol Duc Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

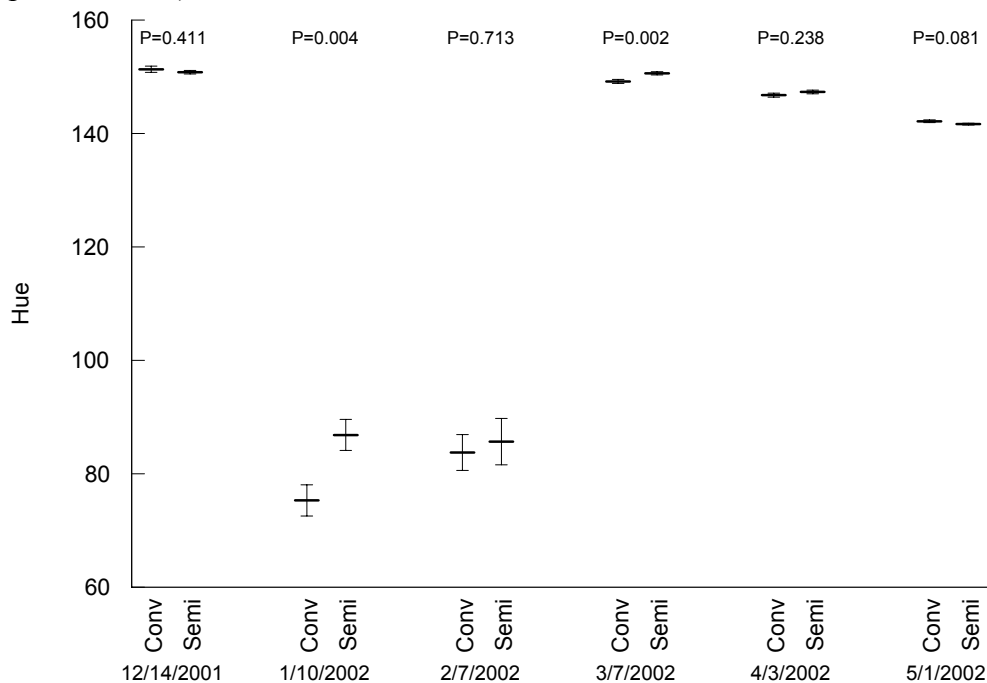


Figure 38. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

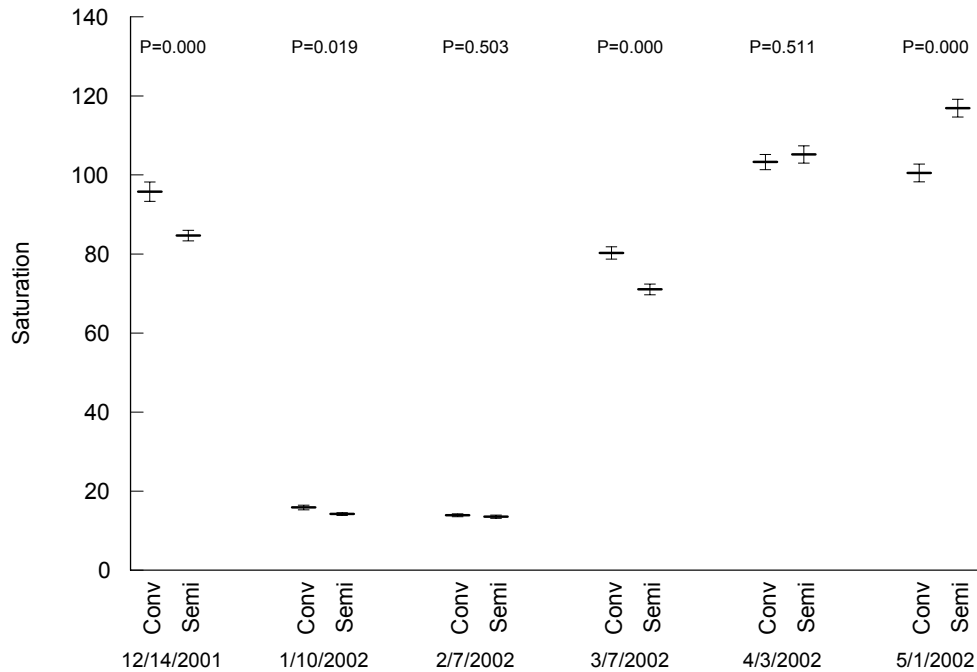


Figure 39. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

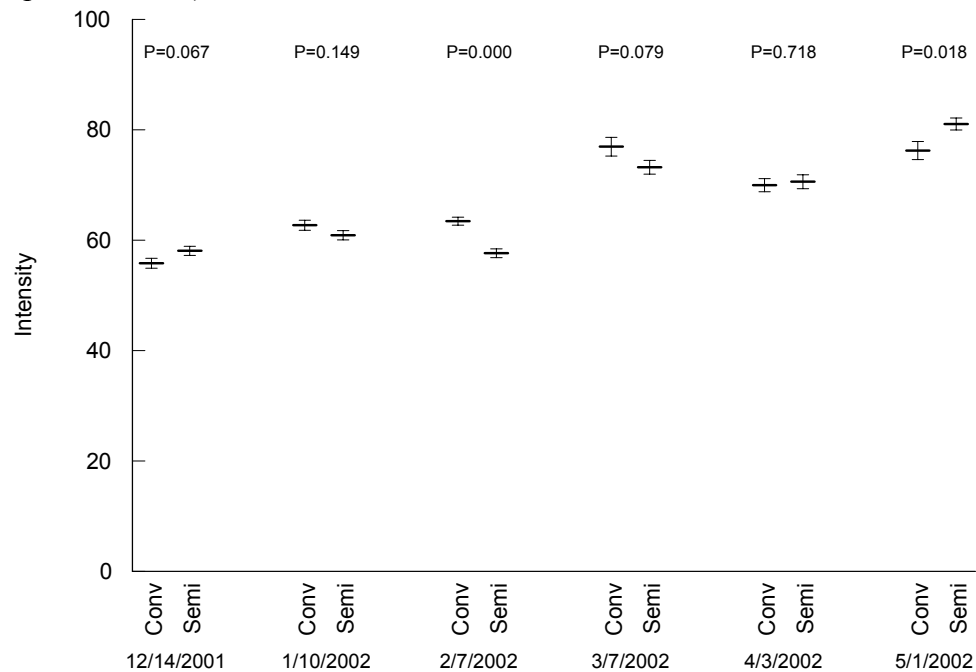


Figure 40. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Minter Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

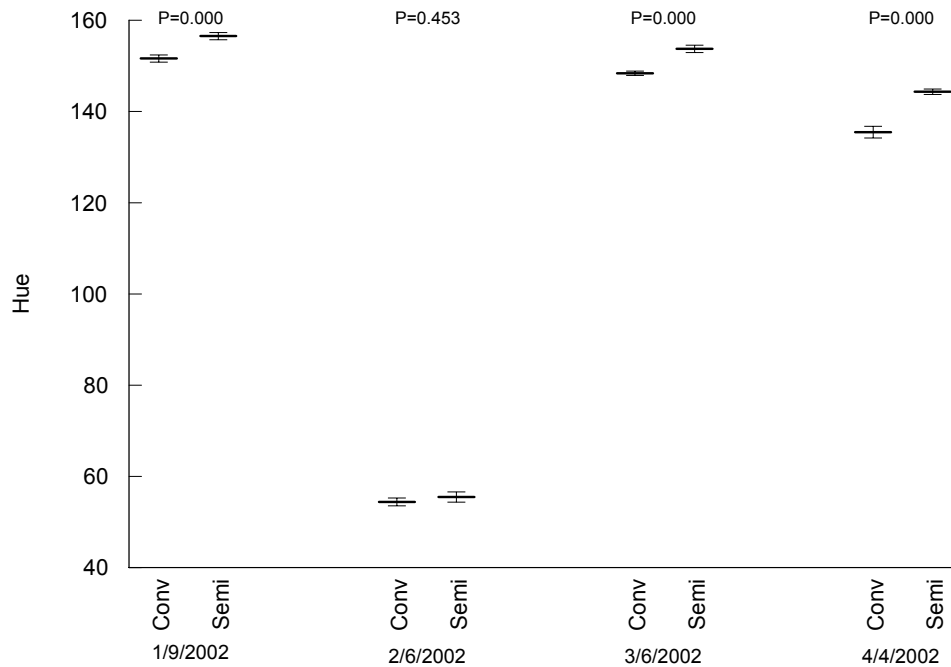


Figure 41. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

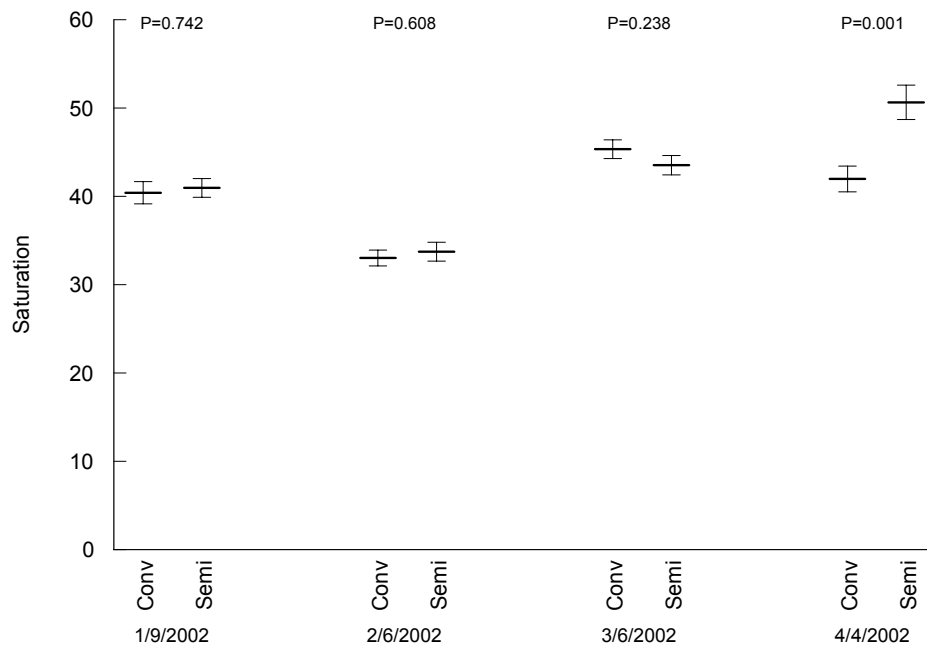


Figure 42. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

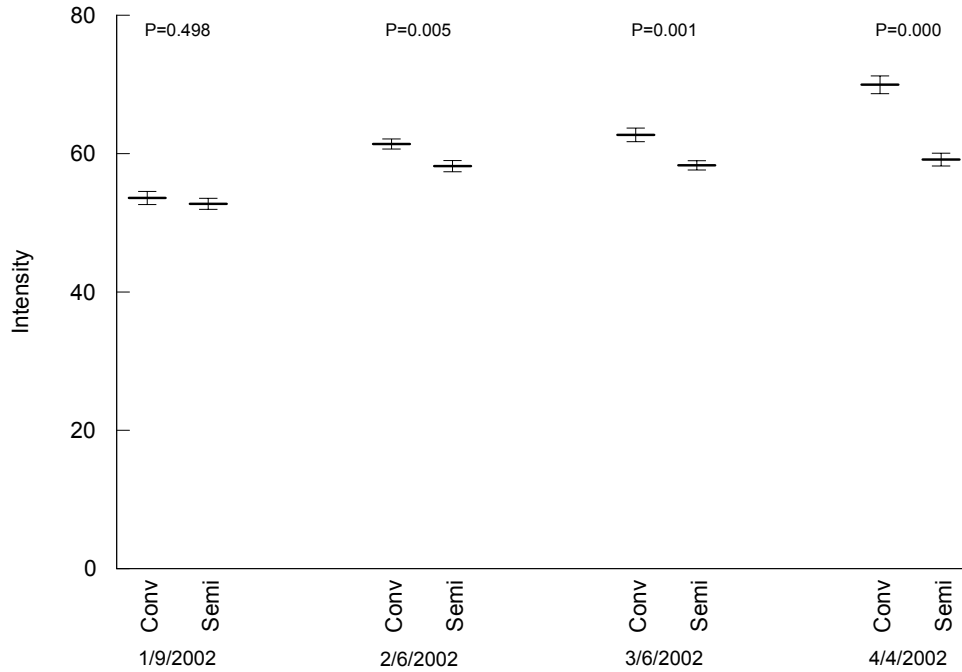


Figure 43. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Soos Creek Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

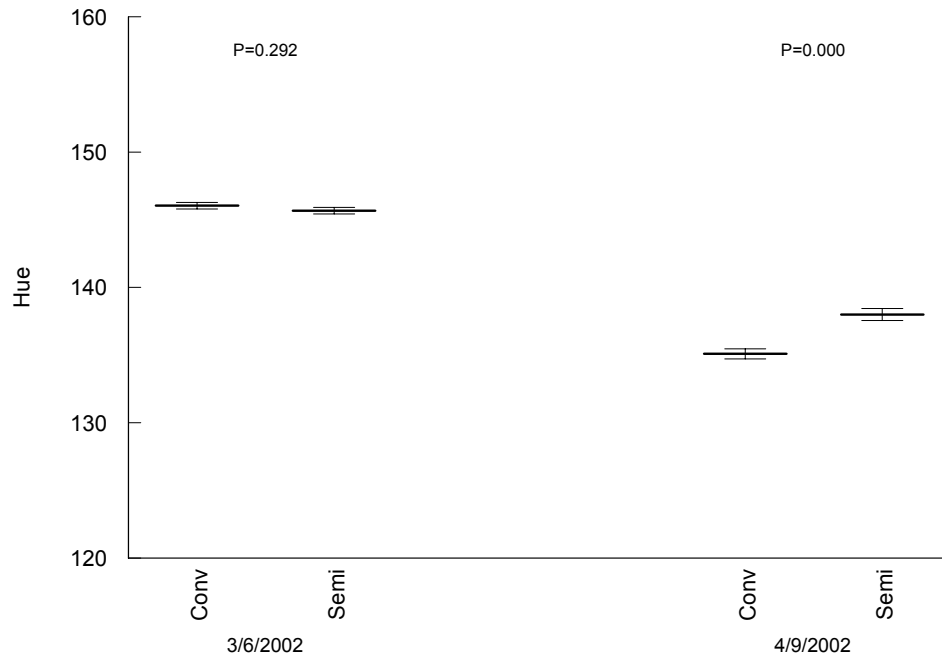


Figure 44. Mean hue values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

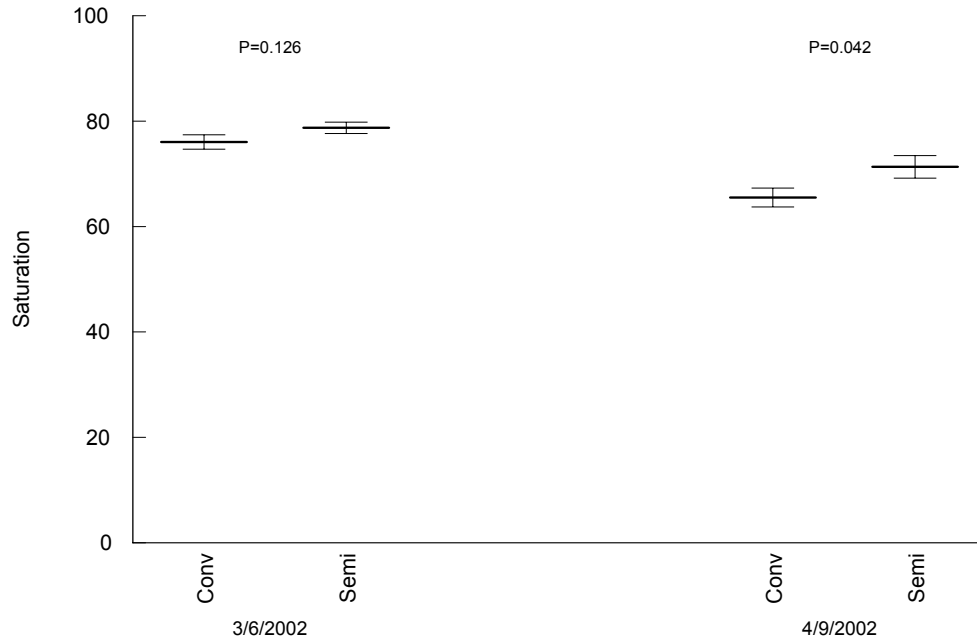


Figure 45. Mean saturation values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

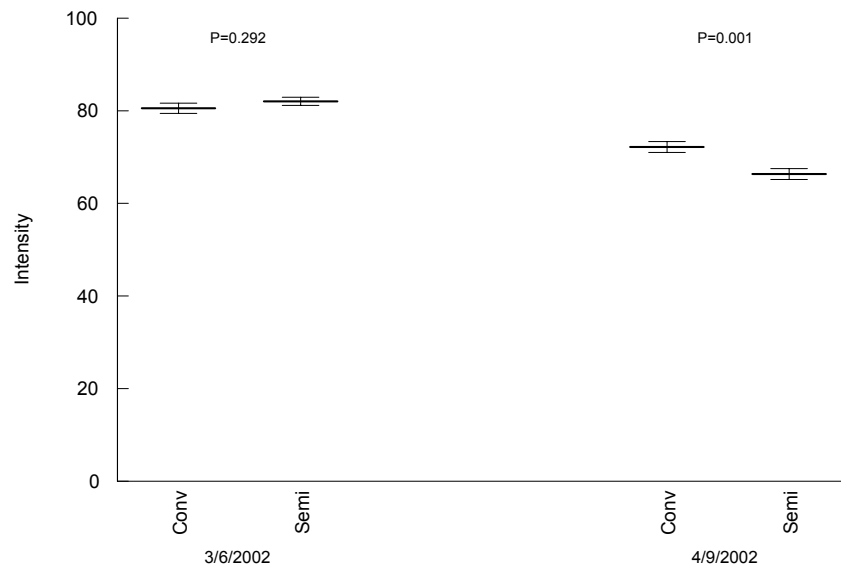


Figure 46. Mean intensity values (with standard error bars) of coho salmon throughout rearing in seminatural (Semi) or conventional (Conv) raceways at Issaquah Hatchery in 2002 (N = 30 per treatment). P values are based on *t*-tests.

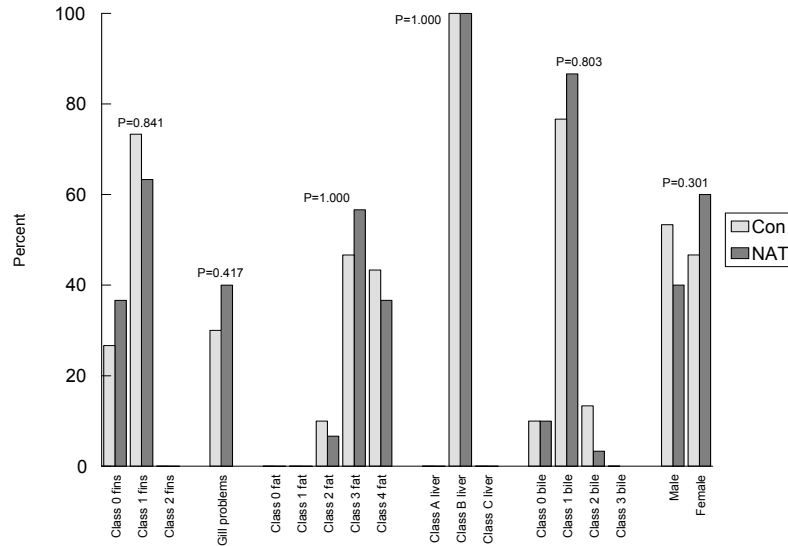


Figure 47. Percentage of coho salmon in different Goede Index classes in the 10 May 2002 Kendall Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

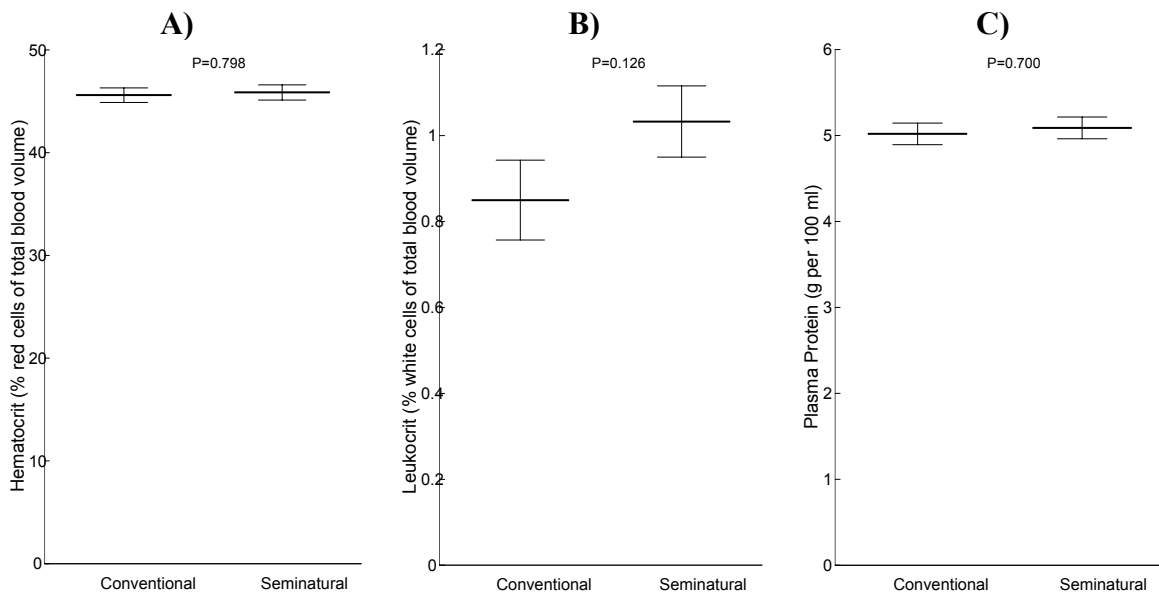


Figure 48. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Kendall Creek Hatchery sampled on 10 May 2002. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 28 conventional and 30 seminatural) P value based on *t*-tests.

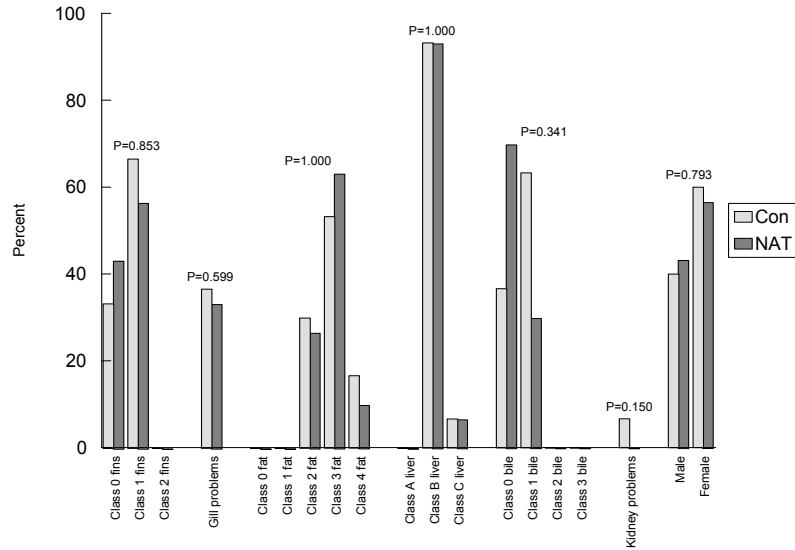


Figure 49. Percentage of coho salmon in different Goede Index classes in the 12 April 2002 Sol Duc River Hatchery fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

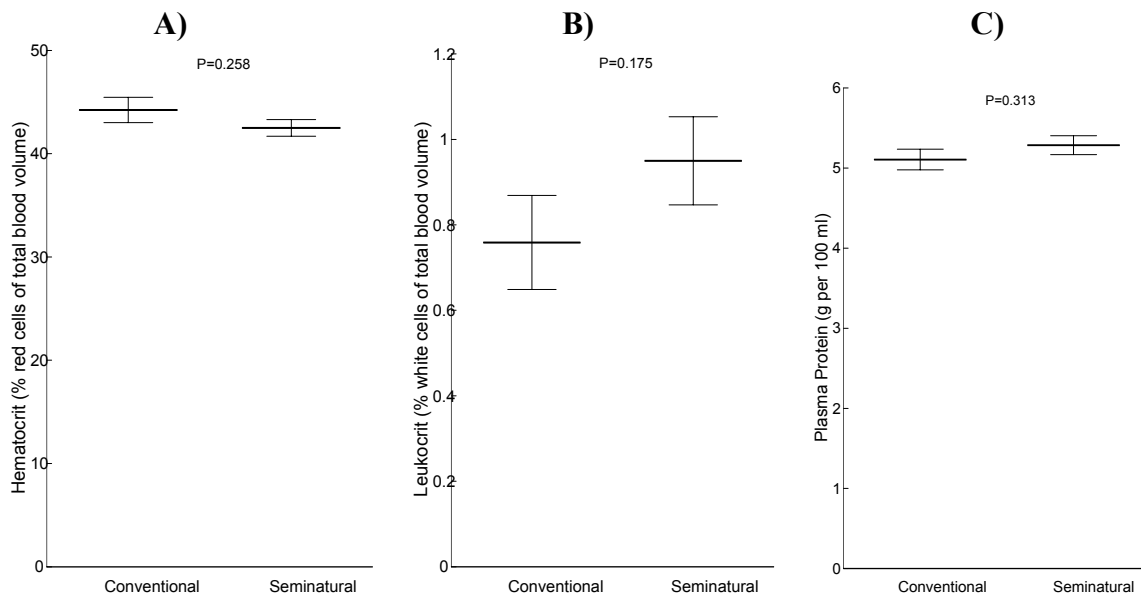


Figure 50. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Sol Duc River Hatchery sampled on 12 April 2002. A) hematocrit and B) leukocrit (N = 29 conventional and 30 seminatural) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 28 per treatment) P value based on *t*-tests.

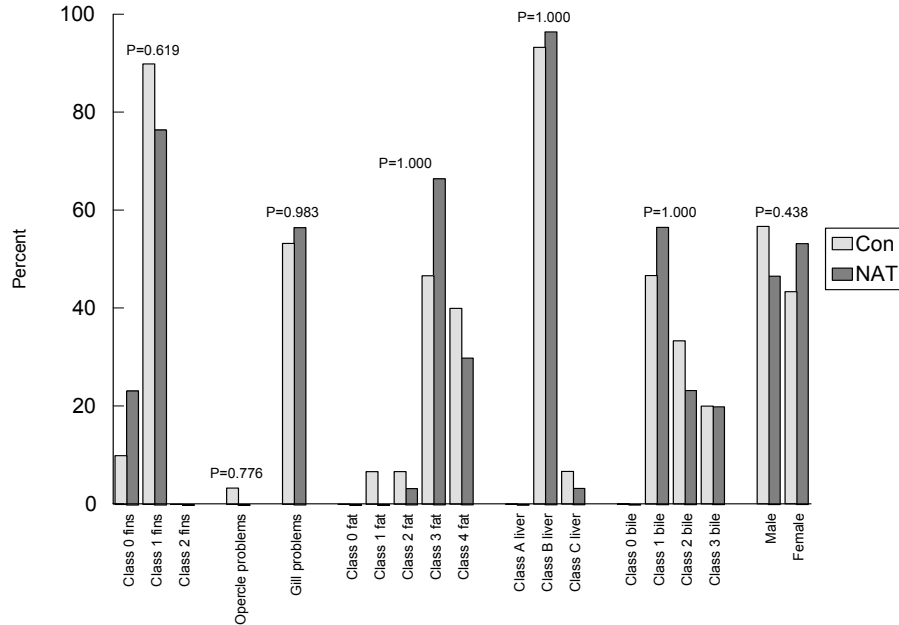


Figure 51. Percentage of coho salmon in different Goede Index classes in the 1 May 2002 Minter Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

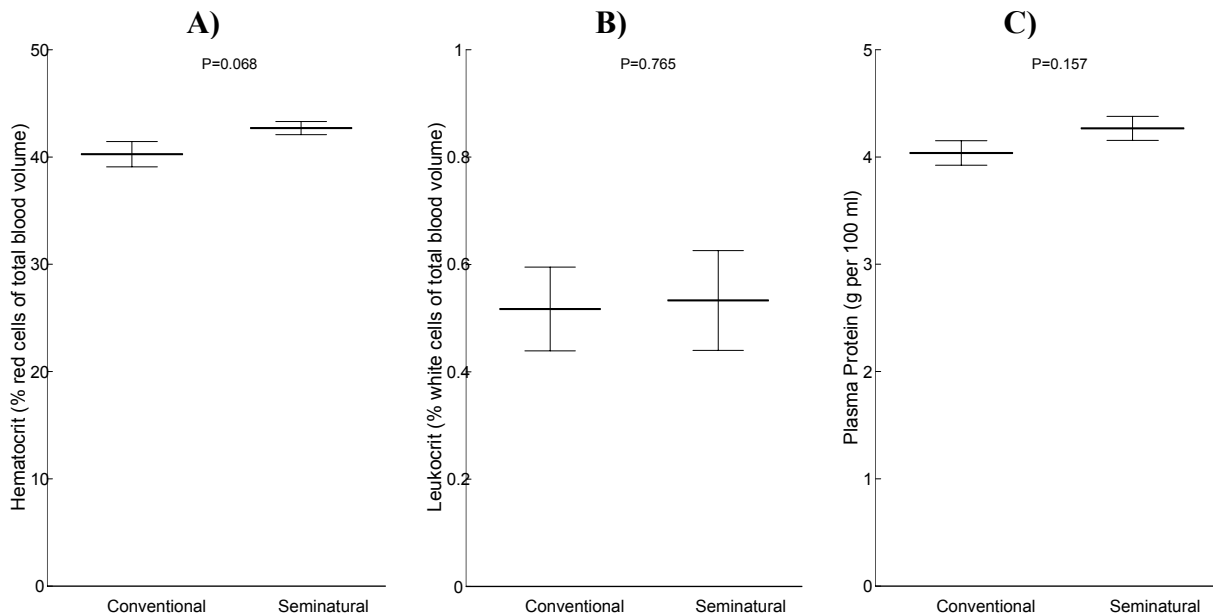


Figure 52. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Minter Creek Hatchery sampled on 1 May 2002. A) hematocrit and B) leukocrit (N = 30 per treatment) P values based on *t*-tests of arcsine transformed data; and C) plasma protein (N = 27 per treatment) P value based on *t*-tests.

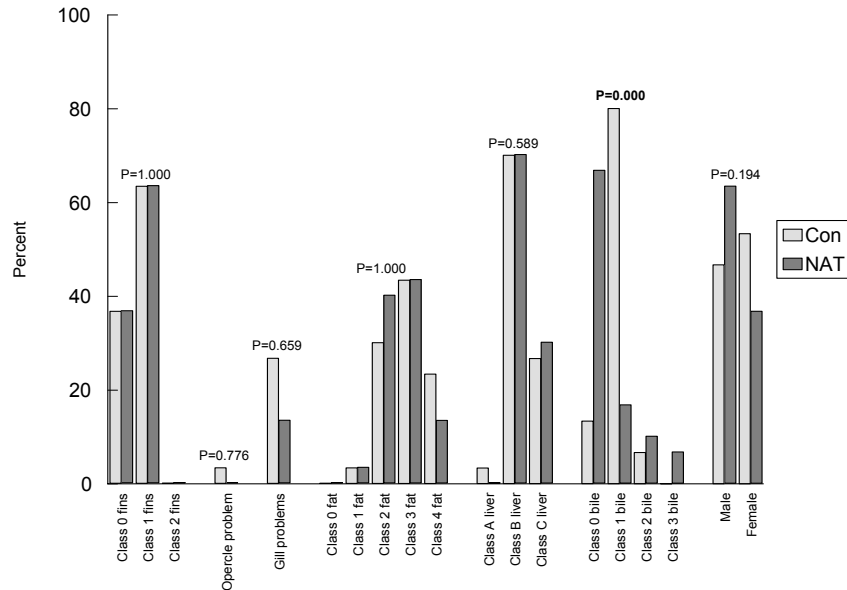


Figure 53. Percentage of coho salmon in different Goede Index classes in the 4 April 2002 Soos Creek fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

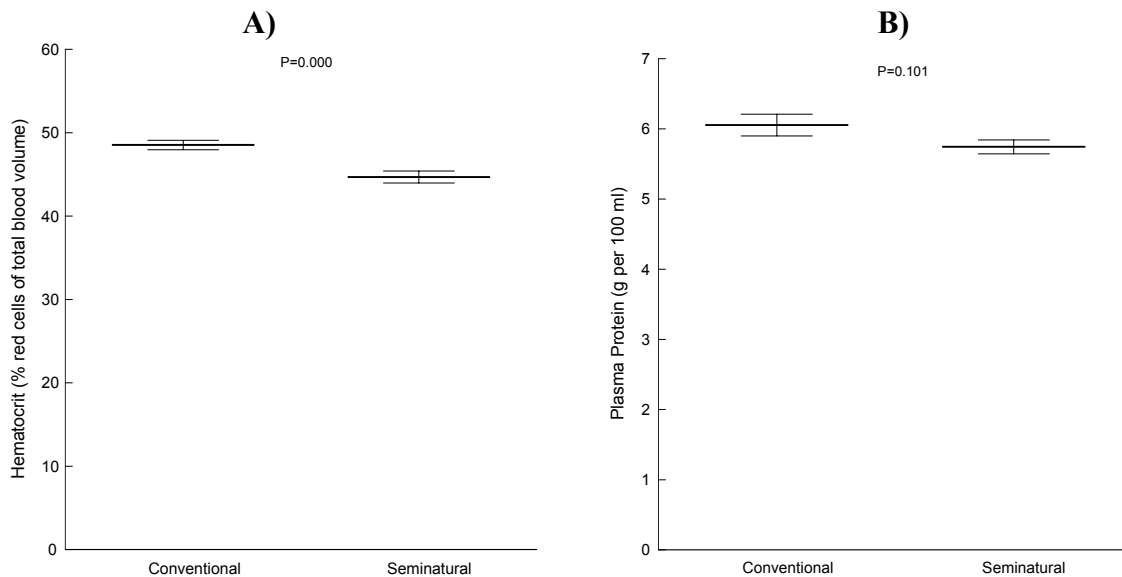


Figure 54. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Soos Creek Hatchery sampled on 4 April 2002. A) hematocrit (N = 30 conventional and 29 seminatural) P value based on *t*-tests of arcsine transformed data; and B) plasma protein (N = 28 conventional and 27 seminatural) P value based on *t*-tests.

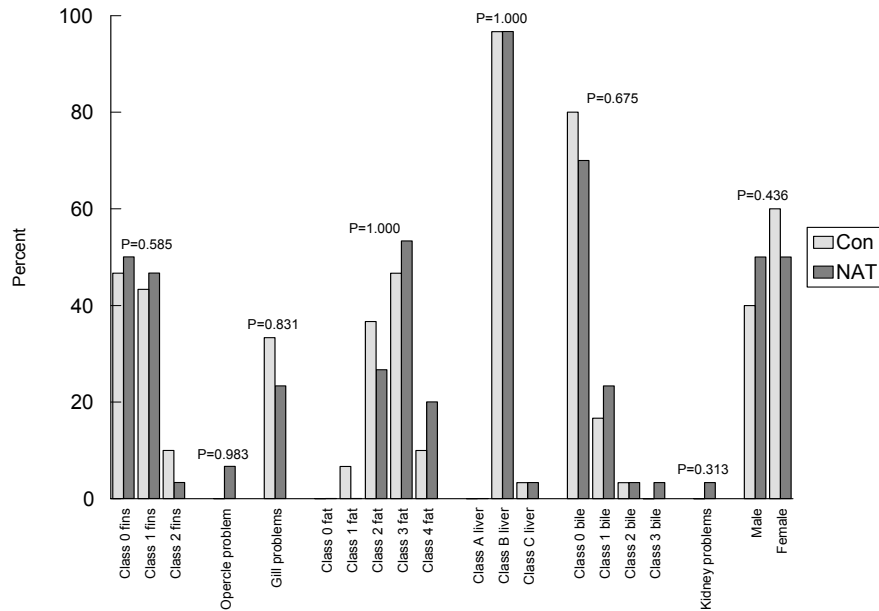


Figure 55. Percentage of coho salmon in different Goede Index classes in the 9 April 2002 Issaquah Hatchery fish condition profile. Fish were reared in seminatural (NAT, N = 30) or conventional (Con, N = 30) raceways. P values are based on contingency table analysis.

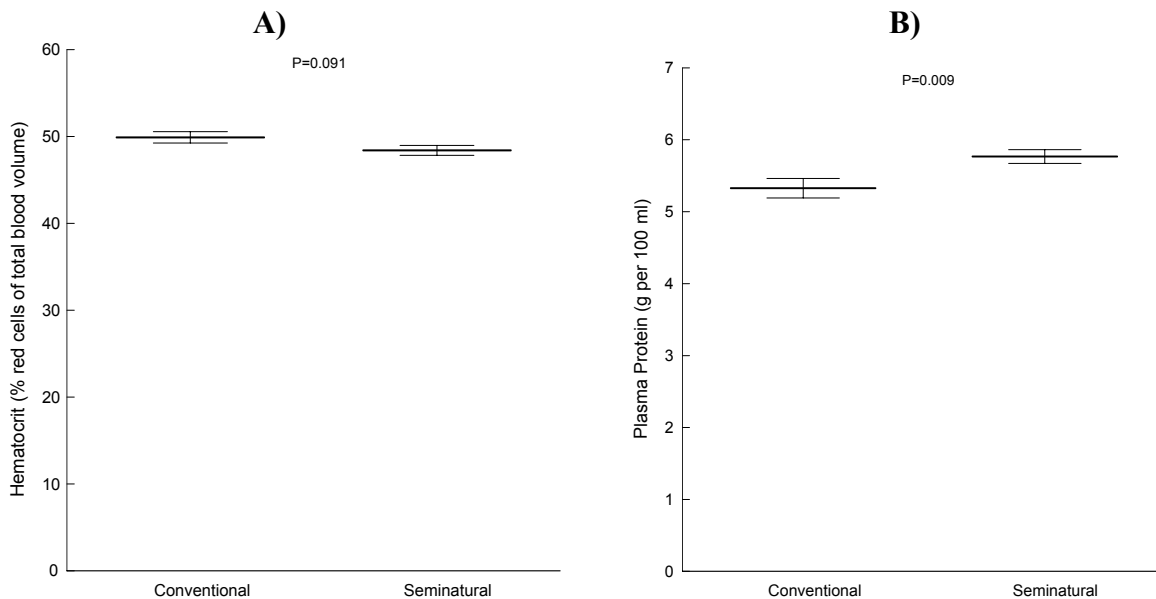


Figure 56. Means (with standard error bars) of blood variables from coho salmon reared in seminatural or conventional raceways at Issaquah Hatchery on 9 April 2002. A) hematocrit (N = 30 conventional and 28 seminatural) P value based on *t*-tests of arcsine transformed data; and B) plasma protein (N = 26 conventional and 30 seminatural) P value based on *t*-tests.

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Section 4

DEVELOPMENT OF SOCIAL BEHAVIOR AND COMPETITIVE ABILITY OF JUVENILE STEELHEAD GROWN IN ENRICHED AND CONVENTIONAL ENVIRONMENTS, AND THEIR COMPETITIVE IMPACTS ON NATURALLY REARED FISH (FINAL REPORT FOR THE PERIOD MARCH 1, 1999 THROUGH FEB 29, 2000).

by

Barry A. Berejikian, E. Paul Tezak, S. Riley, and Anita L. LaRae¹

Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
Manchester Research Station
P.O. Box 130
Manchester, Washington 98353

¹Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon 97027

Introduction

Steelhead (*Oncorhynchus mykiss*) hatcheries in the Pacific Northwest produce and release yearling smolts primarily for harvest augmentation or mitigation for lost habitat. Migrating smolts released from these ‘production hatcheries’ can displace native, naturally produced (wild) conspecifics fish through aggressive interactions and increase the overall levels of aggression in streams (McMichael et al. 1999). Artificially propagated steelhead are often derived from non-local or domesticated stocks (Busby et al. 1996), and are typically released at larger sizes than wild competitors; both these factors can influence the outcome of agonistic interactions and competitive (Abbott et al. 1985, Berejikian et al. 1996). An evolving use of hatcheries in the Pacific Northwest is for conservation of depleted populations. Conservation hatcheries aim to produce fish, which are similar to wild fish in their size, physiology, behavior, and morphology, with the theoretical expectation that producing more wild-like fish will reduce unnatural harmful interactions with wild fish and expose released fish to more natural selection pressures (Flagg and Nash 1999). Releasing fish into their ancestral environments earlier than the smolt stage (e.g., age-0 ‘parr’) might also reduce the opportunity for developmental divergence of hatchery-reared fish from the wild type (Reisenbichler 1997). However, fish released as parr have the potential to compete with wild fish for an extended time period in the freshwater environment.

The Scientific Review Team appointed by the Northwest Power Planning Council has recommended that the design and construction of new salmon and steelhead hatcheries in the Pacific Northwest incorporate enriched rearing environments, including such attributes as submerged structure, overhead cover, and underwater feed delivery systems (SRT 1998). Enriched rearing habitat added to ‘conventional’ (i.e., barren) vessels, may improve the postrelease survival of chinook salmon smolts (Maynard et al. 1996), affect external body coloration (Maynard et al. 1995), and improve the competitive ability of juvenile steelhead (Berejikian et al. 2000). Introductions of hatchery-reared salmonids can disrupt natural social patterns in streams (e.g., Bachman 1984, Nielsen 1994). The ecological implications of producing and releasing juvenile steelhead with enhanced (relative to conventionally reared fish) competitive ability may depend on the nature of their social interactions with wild fish in natural streams.

Steelhead and other salmonids defend contiguous (i.e., adjoining) territories in freshwater streams. Territorial behavior may be an important factor in regulating the abundance of juvenile salmonids in streams to the extent that it functions as a ‘self-thinning’ mechanism (Elliott 1990, Grant et al. 1998). Territory size in steelhead decreases with increasing food abundance and decreasing competitor density, and the territory size requirements for steelhead increase with body size (Keeley and McPhail 1998, Keeley 2000). All these factors being equal, however, the patterns of resource acquisition in different rearing environments (Ryer and Olla 1991, 1995) may have effects on territorial behavior, which persist after the fish are placed into a novel environment (Berejikian et al. 1996). Therefore, the behavior of fish reared in contrasting environments might differentially influence the territory size of wild competitors and perhaps preclude them from scaling their territory size for optimal energetics. Presently, the influence of structurally modified rearing environments have

an unknown effect on the development of territorial behavior of steelhead, whether developmental differences might persist after release, and the impacts on wild fish.

Berejikian et al. (2000) demonstrated that steelhead fry grown in hatchery rearing vessels containing submerged structure, overhead cover, and underwater feeders (i.e., enriched tanks) socially dominated size-matched competitors grown in “conventional” tanks lacking those features. Steelhead grown in the enriched rearing environment also grew faster in a quasi-natural stream channel containing natural invertebrate drift than conventionally reared competitors. The potential proximate causes, such as behavioral or morphological differences, responsible for the competitive asymmetries were not identified. The present study addresses the effects of culturing steelhead in conventional and enriched tanks and a natural stream environment on a morphological character (dorsal fin size), social behavior, and the respective influences of these factors on differences in competitive ability.

Fin erosion is probably the most commonly documented effect among the numerous morphological changes that can be induced by hatchery rearing environments (Bosakowski and Wagner 1994). Fin condition can be affected by numerous factors including temperature (Winfree et al. 1998), density (Wagner et al. 1997), nutrition (Barrows and Lellis 1999), and various water quality parameters, most of which probably mediate the effects of secondary microbial infections. In steelhead (*O. mykiss*), the initial degradation of epidermal tissue is caused largely by aggressive interactions. Steelhead nip their opponents most frequently on the dorsal fin, causing significantly greater damage to epidermis of the dorsal fin than to other fins (Abbott and Dill 1985). Eliminating agonistic interactions (e.g., by growing fish in individual isolation) can virtually eliminate dorsal fin erosion (Winfree et al. 1998), but it is not practical. However, reducing the frequency of attacks each fish experiences may limit fin damage. Previous studies have demonstrated poorer survival of salmonids which have had all, or a portion, of certain fins artificially removed (Johannsson 1981). However, the effects of non-human induced variation in fin quality on survival-related characteristics of fish is unknown.

This study compares age-0 steelhead reared in conventional and enriched tanks (both as described above) and an isolated section of a natural stream. Steelhead reared in the three environments were from the same parent population, incubated in the same environment, and stocked into their respective environments on the same day. Steelhead were sampled from these environments to test the following null hypotheses:

H₀₁. Rearing environment has no effect on dorsal fin quality;

H₀₂. Rearing environment has no effect on competitive ability determined by laboratory trials of social dominance;

H₀₃. Dorsal fin quality, independent of rearing treatment effects, has no effect on dominance ability in dominance trials;

H₀₄. Rearing environment has no effect on territory size and frequency of aggressive behaviors;

H₀₅. The growth of hatchery-reared fry and competing naturally reared fry stocked into a novel stream channel is unaffected by the rearing conditions (conventional vs. enriched) experienced by the hatchery-reared fry.

Methods and Materials

Study population

Eyed eggs were obtained from artificially spawned steelhead from the Skookumchuck River, Mason County, Washington. This hatchery population was derived from the local wild population and spawning protocols have continued to incorporate naturally produced (i.e., wild) steelhead into the spawning broodstock each year. A total of 5,800 eyed eggs were sampled from the artificial spawning of 12 males and 12 females and transported to University of Washington's Big Beef Creek Research Station, near Seabeck (WA) for final incubation in constant 10 °C well water.

Rearing Treatments

Six hundred fifty fish were stocked into each of six, 1.8-m diameter circular tanks (water volume = 1,520 L; flow = 28 L·min⁻¹) on 27 May 1999. Three enriched (EN) tanks contained the tops of two commercially grown Douglas fir (*Pseudotsuga menziesii*) trees that were submerged to provide in-water structure. A double layer of brown and green camouflage netting hung on a circular PVC frame provided approximately 60% overhead shade cover. Each tank was outfitted with an underwater feed-delivery system, which delivered food at the mid-water depth at two locations on opposite sides of the tanks (see Berejikian et al. 2000 for photograph of the tanks). Three conventional (CO) tanks contained no overhead cover or in-water structure (other than a center standpipe), and received 28 L·min⁻¹ of water from above the water surface. Fish in the CO tanks were hand-fed by scattering the food across the surface of the water. Fish in all tanks were fed with equal frequency, which was gradually decreased from about 8 times per day at the beginning of rearing to 4 times per day at the time fish were sampled for the experiments. Ration, as a percentage of biomass, was gradually decreased throughout the rearing period as follows: 4.5% (14 days), 3.5% (13 days), 2.0% (7 days), 1.75% (37 days). All tanks were cleaned weekly.

On the same time emergent fry were stocked into the rearing tanks, 1,950 emergent fry were also stocked into a natural stream channel. To reduce rearing densities to more natural levels, 300 fry were removed on 9 June 1999 and 350 fry were removed on 23 June 1999 by seining. The stream channel consisted of a screened-off section of a side channel of Big Beef Creek, Kitsap County, Washington. The 35-m long section received 0.05 m³/s water flow from springs and the main channel of Big Beef Creek, which was augmented with 0.025 m³/s well water. Red alder (*Alnus rubra*), western cedar (*Thuja plicata*), and salmonberry (*Rubus spectabilis*) comprised the main canopy and streamside vegetation. Some woody debris (12 denuded Douglas fir trees) was added to provide structure and cover from avian and terrestrial predators. No fish predators were present. Steelhead fry were dependent on natural food produced within the channel.

All of the fish in each tank and the stream were marked between 12 and 16 July 1999, with a visible latex tag (Pow'rject System, Newwest Technologies, Santa Rosa, CA:2) injected into the anal fin tissue. Fish in each tank and the stream were marked with a different color. Fish in the three EN tanks received blue, aqua, and red tags, those in the CO tanks received purple, orange and green tags, and naturally reared fish received brown tags.

Dorsal Fin Index

Juvenile steelhead were sampled for dorsal fin condition 27, 50 and 64 days after being stocked in the 6 rearing tanks and the stream environment. On each sampling date, individuals were removed (without conscious selection) from each tank, anesthetized (tricane methane sulfonate), and positioned on their right side for photographs. The dorsal fin was positioned such that the anterior-most fin ray was nearly perpendicular to the dorsal body surface. Lateral images of the fish were captured with a Nikon E3 digital SLR camera. The images were imported into (ImagePro+ 4.0 by Media Cybernetics¹). Digitized measurements were made of standard length (SL), anterior-most dorsal fin ray length (AD), and posterior-most dorsal fin ray length (PD). Dorsal fin index was calculated by the following equation: $((AD \cdot PD) \cdot 2^{-1}) \cdot SL^{-1}) \cdot 100$. Depending on the sampling date, sample sizes ranged between 10 and 15 fish per tank.

Dorsal fin index (DFI) data for the two hatchery rearing treatments (EN and CO) were first analyzed by a nested ANOVA. Individual tanks were nested within hatchery rearing treatment to determine the contribution of random tank effects, not related to rearing treatment. In the absence of significant random tank effects, data were combined among tanks within each hatchery rearing treatment for comparison to the naturally reared fish by a one-way ANOVA.

Treatment Effects on Dominance and Aggressive Behavior

This experiment was conducted to determine whether differences in dominance status and aggressive behavior (of fish of similar dominance status) existed between steelhead fry grown in CO, EN and natural environments. Comparisons of dominance status and aggressive behavior were conducted in a 10-m long by 1.5-m wide flume. Each flume was divided longitudinally with a solid divider, and screens were placed perpendicular to the flow to produce 22, 0.75-m long by 0.75-m wide sections in the flume. The substrate of each section consisted of a 5-cm thick layer of 1.0 to 1.5-cm diameter gravel. The flume received 30 L·min⁻¹ of 12 °C well water, re-circulated at a flow of approximately 1,700 L min⁻¹ by a 2-horsepower submersible pump. Water depth was maintained at 24 cm. A solid bank of wide-spectrum florescent lights on a simulated photoperiod of 16 hours light to 8 hours dark provided light. The side walls of the flumes consisted of double-paned glass, which allowed complete viewing of all fish in each cell. Live *Daphnia* were continuously introduced into the head box of each flume by peristaltic pumps at a rate of approximately 30 g d⁻¹ wet weight (approximately 470 individuals h⁻¹). The *Daphnia* entered each section through the upstream screen, such that within each cell, fish in the upstream-most position had first access to the food.

2 Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA

Three fish, one fish from each of the three treatments, were matched for equal body weight (within 7.5%) and introduced into each of one section of the flume. After 48 hours acclimation, the frequency of attacks (including nips, charges, and chases) and lateral displays were recorded for each fish in the cell for 10 min. A fish was considered dominant only if it 1) maintained its feeding station until the end of the observation, 2) delivered more attacks than it received, 3) never exhibited submissive posture (Keenleyside and Yamamoto 1962), and 4) never retreated when attacked or approached by other fish. At the end of the day one, each dominant fish was removed and assigned a rank of three. The observation procedure was repeated on day two when the dominant fish was assigned a rank of two, and the remaining fish was assigned a rank of one. A total of 90 trials were conducted, including an equal number of contests between all combinations of the three rearing tanks per treatment and naturally reared fish. In three trials, one of the fish was either not visible (one trial) or found dead on one of the observation days, so the results of 87 trials are presented. A Wilcoxon signed rank test (Z – approximation, Zar 1984) was used to compare the ranks of the three treatments.

Fin Quality Effects on Dominance and Aggressive Behavior

The effects of fin quality on competitive ability (dominance) were evaluated independently of potential rearing treatment effects. Fish grown in a common 1.5-m diameter tank were sampled, weighed, and sorted into weight categories of $\leq 5\%$ difference. Within each weight category, replicate pairs were created within weight categories in which the two fish differed in dorsal fin quality. A fish with a subjectively judged ‘high’ quality dorsal fin was paired with a fish with a relatively ‘low’ quality fin. The pair was introduced simultaneously into one section of the flume and allowed to acclimate and compete for 20 hours prior to behavioral observations, which were conducted as in the previous experiment. After observations were complete, fish were removed and photographed as described above, and DFI was calculated. A total of 56 trials were conducted. Unfortunately, digital files containing lateral images from 18 of the trials were not recoverable, so we report the outcomes of 38 dominance trials in which we have quantified fin quality data, and 18 trials in which only relative fin quality determinations were available. A Sign Test compared the proportion of dominant fish from the two groups.

Territorial behavior

This experiment was conducted to test the null hypothesis that no differences in territorial behavior exist between steelhead grown in EN, CO, or natural environment. Specifically, we quantified aggressive interactions and spatial distributions of within-treatment groups. The experiment was conducted in another flume identical to the one described for the dominance experiment, except that individual sections measured 2.25 m long by 2.25 m wide. A 10-cm square grid was positioned on the substrate of each section so the location of individuals could be monitored by a video camera set above the flume. The camera position and recording functions were controlled from a remote location to minimize disturbance to the fish.

To begin each trial, nine fish from a single treatment were matched for body weight (within 10% difference), and introduced into a single flume section. After a 24-

hour acclimation period, the behavior of fish in each of the six flume sections was recorded on video tape for 30 minutes each day for 2 days. The allocation of treatments to flume sections and the order of video recording was randomized. A total of 30 trials (10 per treatment) were conducted over a 3-week period beginning in mid-July.

Behavioral frequencies were also quantified by direct observation for three 10-min periods during each trial. A territory was defined as the area defended around a feeding station. A more precise definition for the purposes of this study were developed during a set of pre-trial observations. Three aggressive behaviors (bites, chases, and displays) were recorded for each territory holder, and the number of displacements involving the removal of a territory-holder by an intruder were also recorded. The positions of each territory holder were mapped (using the 10-cm grid) at the beginning and end of each video recording session to determine the mean distance between territories, and the total number of definable territories. Site fidelity was determined by measuring the area of movement surrounding a territory (territory size), and the duration of time spent away from it. The frequency of aggressive behaviors, territory distance, territory size and duration were compared among treatments by one-way ANOVA.

Treatment effects on growth of hatchery-reared and naturally reared fish

The growth experiments were conducted in a 45-m long by 6-m wide outdoor stream channel. The side-walls and bottom of the channel are constructed of concrete at a constant 3% gradient. Well water was supplied at $80 \cdot \text{L} \cdot \text{min}^{-1}$, and recirculated by three submersible, 2-horsepower pumps at a flow of approximately $5,100 \cdot \text{L} \cdot \text{min}^{-1}$. Temperature was maintained between 11.2 and 15.0 °C throughout the experiment, with a diurnal fluctuation of ~ 2 °C. A wooden barrier divided the stream along its entire length into two side-by-side channels. The channel was further divided by seven wire mesh (3.0-mm opening) screens set on top of weirs situated across the channel, perpendicular to the flow. This configuration created 16 replicate, 5.0-m long by 3.0-m wide sections in the channel. The substrate consisted of 3- to 5-cm diameter gravel, graded to create as similar a depth and velocity profile as possible among the 16 separate sections. Algal growth on the substrate and sidewalls supported abundant aquatic insect populations (Family: *Chironomidae*), so no artificial feeding was necessary. No piscine predators were introduced, and a single layer of 2.5-cm square mesh netting placed over the entire channel excluded avian predators. An underwater viewing chamber positioned alongside the entire length of one of the 16 sections allowed for general observations of steelhead behavior. The experimental design outlined below was used to test the following null hypotheses:

H_{05a}: Fish reared in EN tanks, in competition with smaller naturally reared (NR) fish, grew at the same rate as conventionally reared fish in competition with smaller NR fish;

H_{05b}: The growth of NR fish did not differ depending whether they were stocked into the channel with EN or CO fish;

H_{05c}: The total increase in biomass of channel sections was the same for sections containing NR + CO and NR + EN.

Seven of the 14 sections simultaneously received 33 EN fish (11 from each of the 3 rearing tanks) and 15 stream-reared fish, and the other 7 sections simultaneously received 33 CO fish (11 from each of the 3 rearing tanks) and 15 stream-reared fish. All fish were stocked on 6 August 1999 and removed by seining either 32 or 33 days later. Upon removal, each fish was identified to rearing tank by anal-fin tag color, measured (FL), and weighed (nearest 0.01 g). The overall mean (\pm s.d.) weights (g) of EN fish (1.33 ± 0.06), and CO treatment (1.32 ± 0.8) were significantly greater ($P < 0.05$) than that of NR fish (0.94 ± 0.10) at the time they were simultaneously stocked into the stream channel.

Instantaneous growth rates (IGR; change in wet weight ($\text{g} \cdot \text{d}^{-1}$)) were calculated using the following formula:

$$\text{IGR} = (\log_e w_2 - \log_e w_1) \cdot \Delta t^{-1}$$

where w_1 is the mean initial weight of fish within a stream channel section, w_2 is the mean final weight of fish within a stream channel section, and Δt is the duration in days between stocking and removal in that section.

To test H_{05a} , data was analyzed by a single factor ANOVA with rearing treatment (EN vs. CO) as the main effect and the difference in weight between the treatment fish and NR fish (within each section) as the covariate. To test H_{05b} , data were analyzed by ANCOVA with treatment (EN vs. CO) as the main effect and the difference in weight between the treatment fish and NR fish (within each section) as the covariate. To test H_{05c} , data were analyzed by ANCOVA with rearing treatment as the main effect and beginning total biomass as the covariate.

Results

Dorsal Fin Index

No significant differences in DFI among the two hatchery rearing treatments on existed on day 27 (main effect: $F_{1,57} = 0.801$, $P = 0.375$; nested tank effect: $F_{4,57} = 1.76$, $P = 0.15$). The nested (tank within treatment) effect was significant on day 50 ($F_{4,54} = 7.79$, $P < 0.01$), when one of the CO tanks had a lower mean DFI (Tukey's HSD, $P < 0.05$) than the other two (between which mean DFI did not differ significantly). On day 64, fish grown in the EN treatment had a significantly higher mean DFI than fish grown in the CO treatments (main effect: $F_{1,83} = 182.18$, $P < 0.001$); nested effect: $F_{4,83} = 1.92$, $P = 0.115$).

In comparing the two hatchery rearing treatments to naturally reared fish, no significant differences existed on day 27 ($F_{2,96} = 0.776$, $P = 0.463$). However, there was a difference in DFI among treatments on days 50 ($F_{2,70} = 26.13$, $P < 0.001$) 64 ($F_{2,80} = 34.60$, $P < 0.001$). The conventionally reared fish had a lower mean DFI than both the EN and NR fish on day 50 and day 64 (Fig. 1). The CO tank, which had a significantly lower DFI than the other two tanks in that treatment on day 50 was removed from this analysis before comparing all three rearing treatments. Therefore, the among-treatment

comparison was conservative with respect to the potential for committing a Type I statistical error.

Dominance and aggressive behavior

Fish grown in the EN tanks and natural stream had similar dominance ranks, but both of these treatments had significantly greater dominance ranks than fish grown in the CO tanks (Table 1). On the first day of the experiment, dominant fish from the EN tanks ($n = 36$), CO tanks ($n = 19$) and the natural stream ($n = 32$) differed in the frequency of displays ($H = 11.56$, $P = 0.003$; Fig. 2b), but did not differ in the frequency of aggressive attacks ($H = 4.14$, $P = 0.126$; Fig. 2a) they elicited towards the other two competitors in a given cell.

In a commonly reared group of fish, dorsal fin quality did not have a significant effect on dominance (Table 2). Fish with relatively high dorsal fin quality established dominance in 22 of 38 (57.8%) of the contests against fish with relatively low dorsal fin quality ($P > 0.50$). In the 18 trials in which dorsal fin quality could only be subjectively rather than digitally evaluated, the results were similar to those in which the digitized measurements were made. Fish with high quality fins established dominance in 10 of 18 (55.5%) of trials against fish with low quality fins.

Treatment effects on growth of hatchery-reared and naturally reared fish

The Instantaneous growth rates of CO and EN fish did not differ significantly after approximately one month of rearing in the quasi-natural stream channel. ($F_{1,11} = 2.78$, $P = 0.124$). The instantaneous growth rate of NR fish did differ depending on whether they were stocked into the channel with EN or CO fish ($F_{1,11} = 3.328$, $P = 0.095$). The weight difference (covariate) between the hatchery reared fish and the stream reared fish at the time of stocking had no effect on growth of hatchery reared fish ($F_{1,11} = 1.33$, $P = 0.273$), or naturally reared fish ($F_{1,11} = 0.54$, $P = 0.477$). The overall change in biomass (i.e., NR + CO vs. NR + EN) was unaffected by hatchery the rearing treatment (main effect: $F_{1,11} = 0.70$, $P = 0.42$) and initial biomass (covariate: $F_{1,11} = 0.274$, $P = 0.611$).

Discussion

Steelhead fry grown in EN rearing tanks and naturally reared fry were socially dominant and had larger dorsal fins than fry grown in the CO tanks. Despite the observed competitive inferiority of CO fish in dominance trials, both EN and CO fish grew at similar rates in competition with NR fish in a quasi-natural stream channel, and the growth rate of NR fish did not differ in the presence of EN vs. CO fish.

Fish grown in the EN tanks exhibited dorsal fin quality similar to that of NR fish, and greater than that of CO fish at an early age (50 d post-emergence). Bosakowski and Wagner (1994) documented reduced fin erosion in rainbow trout grown in tanks containing a rugose (e.g., cobble) substrates. In the present study, however, the CO and EN tanks had similarly smooth bottoms, eliminating substrate as a causative factor for

differences between those two treatments. The relative effects of the three variables that differed between the CO and EN tanks (i.e., overhead cover, submerged structure and underwater feeders) could not be determined by our experimental design. Studies of other salmonids have demonstrated that structure in rearing environments can reduce the occurrence of agonistic interactions. We speculate that the visual isolation provided by the submerged structure likely reduced the frequency of nips received by individuals, which occur primarily on the dorsal fins of competitors (Abbott and Dill 1985). Damage to the dorsal fins may have been reduced as a result the presumably reduced nipping frequency.

Fish grown in the EN tanks and natural stream had similar mean dominance ranks when compared to one another, but both groups ranked significantly higher than conventionally reared fish. The differences between EN and CO fish are consistent with the results of Berejikian et al. (2000), in which steelhead fry grown in the same EN and CO treatments were compared in the absence of NR fish. The differences in fin condition appear insufficient to explain the competitive asymmetries among the treatments. In the commonly reared group of fish, those with high quality dorsal fins did not win significantly more contests (56%) than fish with lower quality fins. In comparing the social status among treatments, however, EN and NR treatments had significantly higher dominance ranks in 70% and 66% of trials respectively. In work by Berejikian et al. (2000), EN fish established dominance in 68% of contests against CO fish. The lack of a significant effect of fin quality on dominance does not necessarily imply that fin quality might not be important in determining social interactions or general fitness of fish in more diverse natural environments or over longer time periods. Nevertheless, among-treatment differences in fin condition was insufficient to completely explain the differences in social status.

We compared behavioral frequencies of fish from the three treatments, which had equal social status (i.e., they were all dominant on the first day of the trial). No differences were found in the frequencies of aggressive ‘attacks’, which include nips, charges, and chases. Both the EN and NR fish exhibited higher frequencies of threat displays than CO fish. Threat displays may function both offensively and defensively (Keenleyside and Yamamoto 1962). Although the frequency of threat displays is probably less an indicator of dominance ability than nipping frequency (Holtby et al. 1993), they may function to settle contests with a lesser degree nipping and, therefore, injury. Our data suggest that the CO rearing environment caused a decrease in the frequency lateral displays relative to the wild type (stream-reared fish). However, no decrease was noted for EN fish, providing the first evidence that the development of agonistic behavior may be more natural in EN rearing environments than in CO rearing environments.

Smaller NR fish grew at rates similar to both hatchery treatments (Fig. 3). The growth of NR fish, and total biomass was not affected differentially by the presence of EN versus CO fish even though EN fish were more competitive than CO fish in dominance trials. Other salmonid studies concurrently evaluating behavioral measures of social dominance in the laboratory and growth in simulated stream environments have demonstrated both consistent (Berejikian et al. 2000) and inconsistent (Garcia de Leaniz

1997) results between the laboratory behavioral trials and growth evaluations in stream channels. Recent studies on coho salmon have compared hatchery reared fry to naturally reared fry for dominance ability in the laboratory (Rhodes and Quinn 1998) and growth and survival in a natural stream (Rhodes and Quinn 1999). Rhodes and Quinn (1998) concluded that hatchery reared fry, reared in a conventional manner, were socially dominant to naturally reared fry in laboratory aquariums. In a natural stream, hatchery-reared fry survived at a similar rate, but gained more weight during the summer than naturally reared fry, which were 10% smaller at the time of stocking (Rhodes and Quinn 1999). These findings are contradictory to those in the present study and may reflect differential responses of the two species, coho salmon and steelhead, to hatchery and natural environments.

We suspect that food availability in our stream channel was too high for the competitive asymmetries between the treatments observed in the dominance trials to be reflected in differential growth rates in the stream channel. Increased food availability, at levels above that typical of natural streams, decreases territory size in juvenile steelhead (Keeley 2000) and reduces the frequency of agonistic behaviors (Berejikian et al. 1996). Although invertebrate drift abundance was not directly measured in the stream channel during the month-long growth experiment, the high growth rates in the stream channel probably reflect artificially high food abundance. Fish from all treatments grew approximately 4% per day (body weight), approximately quadrupling their body mass in 1 month, which is a much higher growth rate than has been reported for steelhead fry in natural streams (c.f. Hume and Parkinson 1987, Harvey and Nakamoto 1997). Future studies testing effects of hatchery-wild fish interactions might benefit from evaluations at varying (or natural) levels of food availability.

Whether the release of hatchery-reared fish exhibiting enhanced competitive abilities (e.g., fish reared in enriched tanks), will harm efforts to maintain or rebuild native populations may depend on the potential for two main categories of ecological interactions: 1) competitive interactions between released hatchery-reared fish and natural-origin recruits; and 2) competitive interactions between the released hatchery-reared fish and fish produced from non-indigenous hatchery strays. Interactions will vary depending on fish density, with more intense interactions occurring at densities near carrying capacity. Regarding the first interaction, wild fish should perhaps be considered more valuable than released hatchery-reared fish because they are products of naturally spawning adults, and have not undergone either directional or relaxed selection imposed by artificial propagation (see Waples 1999). Increasing competitive ability of hatchery reared fish through EN rearing habitats may decrease the productivity of the natural-origin-recruits. However, it not be detrimental if the behavior patterns fish reared in enriched environments, including dispersal, migration, habitat use, and social behavior deviate from those of wild fish less than do the behavior patterns of conventionally reared fish. Our results begin to suggest that EN rearing may reduce the developmental divergence of hatchery and wild fish.

Regarding the second interaction, reduction in natural production has resulted from the stocking of domesticated hatchery fish (coho salmon: Nickelson et al. 1986), although competition between offspring of hatchery and wild populations has not been

empirically demonstrated as the mechanism decreasing the production of wild populations. Genetically-based differences in aggressive behavior between hatchery and wild populations (Swain and Riddell 1990, Berejikian et al. 1996, Eium and Fleming 1997) have raised concerns that progeny of non-native hatchery strays may outcompete progeny of wild fish. If hatchery-reared juveniles released for conservation purposes acquire increased competitive ability through rearing in enriched habitats, they may be better able to compete well and establish territories in the presence of invading progeny of non-local hatchery strays.

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Table 1. Results of the dominance experiment comparing steelhead reared in a natural stream (NR), and in conventional (CO) and enriched (EN) hatchery environments in 87 replicate trials. In the ‘comparison’ column, the treatment which ranked higher in the greater number of trials is listed first. Wilcoxon signed ranks test (normal approximation: Zar 1984) was used to compare the treatments.

Comparison (high rank vs low rank)	Higher rank ¹	Lower rank ¹	Z approx.	P
EN vs NR	48 (0.55)	39	1.10	0.269
EN vs CO	61 (0.70)	26	3.93	< 0.001
NR vs CO	57 (0.66)	30	2.77	0.006

1/ Refers to the treatment listed first in the comparison column

Table 2. Outcomes of dyadic dominance challenges between fish with high and low dorsal fin indices (relative to their competitor). The mean (+ s.d.) attacks, displays and food strikes are compared between dominant fish possessing high quality fins (n = 22) and dominant fish possessing low fin quality (n = 16).

Trait	Relative Dorsal Fin Index		P value
	High	Low	
Number of winners (%)	22	16	0.50
DFI (s.d.)	10.85 (1.32)	8.25 (1.53)	< 0.001
Fork length (cm)	4.22 (0.29)	4.19 (0.29)	> 0.50
Attacks	4.49 (7.30)	6.94 (17.40)	>0.50
Displays	0.17 (0.48)	0.19 (0.54)	>0.50
Food strikes	25.04 (15.45)	21.81 (19.74)	>0.50



Figure 1. Mean (± 2 S.E.) dorsal fin index of age-0 steelhead reared in conventional and enriched tanks and a natural stream channel.

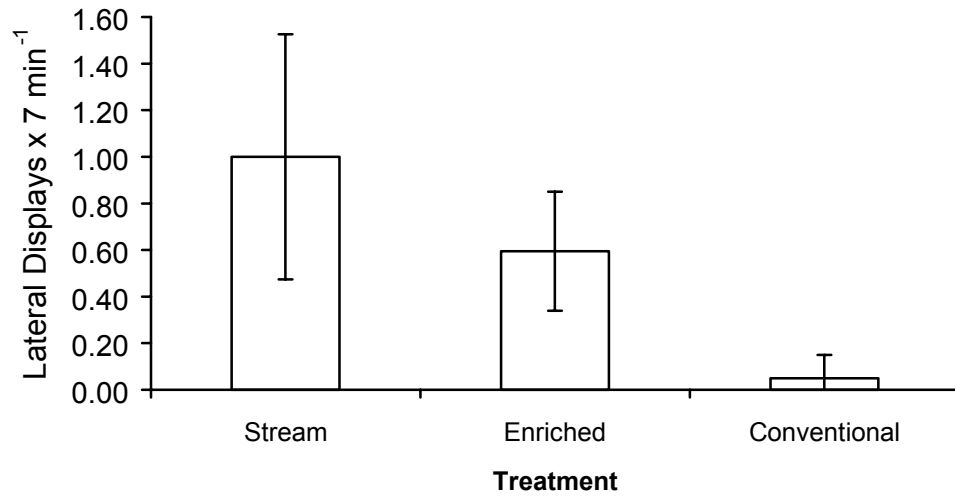
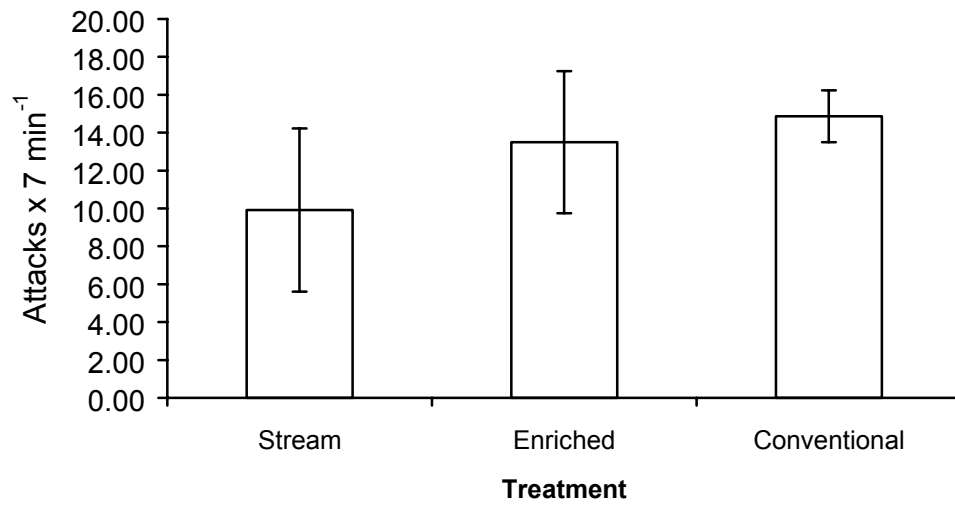


Figure 2. Mean (+ 2 S.E.) number of attacks (upper graph) and threat displays (lower graph) elicited by fish reared in the enriched and conventional tanks, and the natural stream channel. Data includes fish from each treatment that were dominant on day 1 of the experiment.

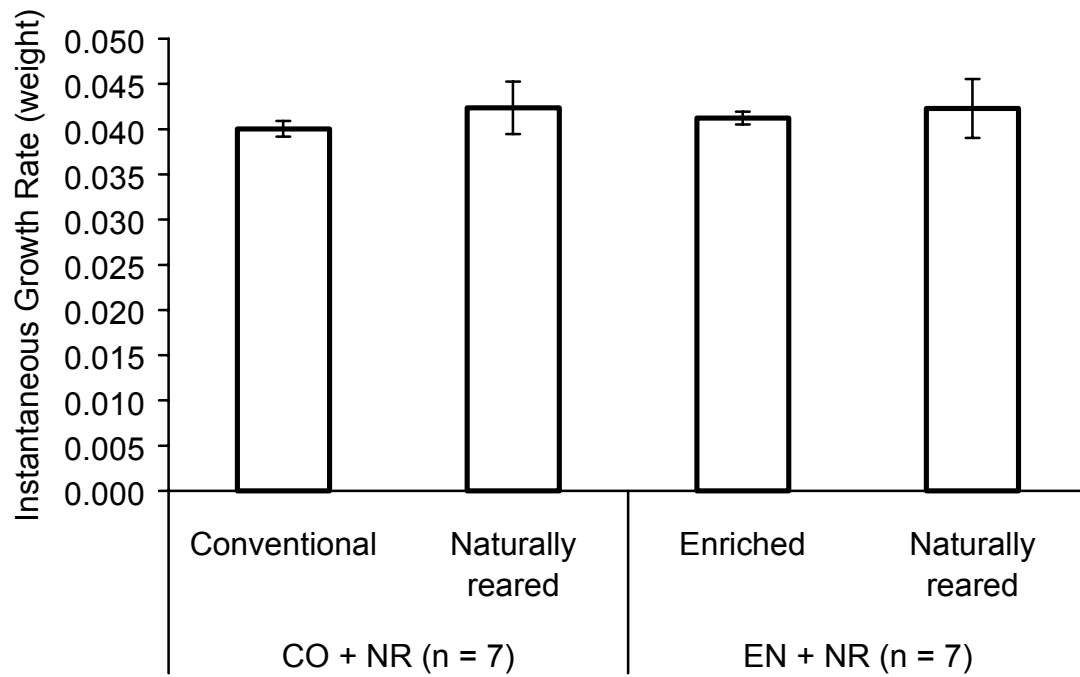


Figure 3. Mean (+ 2 S.E.) instantaneous growth rate of fish reared in conventional and enriched tanks and the natural stream. Seven stream channel sections contained fish reared in the stream and conventional tanks, and the other seven sections contained fish reared in the stream and enriched tanks.

Section 5

FACTORS AFFECTING ACQUIRED PREDATOR RECOGNITION, FRIGHT RESPONSE, AND PREDATOR AVOIDANCE IN HATCHERY-REARED CHINOOK SALMON (PROGRESS REPORT FOR THE PERFORMANCE PERIOD: 1 MARCH 2000 THROUGH 28 FEBRUARY 2001)

by

Barry A. Berejikian, Anita L. LaRae¹, and E. Paul Tezak

Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
Manchester Research Station
P.O. Box 130
Manchester, Washington 98353

¹Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon 97027

Introduction

Among other hatchery reforms, anti-predator conditioning has the potential to improve the postrelease survival of hatchery-reared anadromous salmonids. Predator avoidance differences between hatchery and wild juvenile steelhead (*Oncorhynchus mykiss*) have a genetic basis (Johnnson and Abrahams 1991, Berejikian 1995). However, within populations, the ability of hatchery-reared juvenile salmonids to avoid predation improves with experience. Laboratory studies have demonstrated that anti-predator behavior and predator avoidance ability of juveniles of several salmon species increased following brief exposure to predators (Patten 1977, Olla and Davis 1989, Berejikian 1995, Healey and Reinhardt 1995). However, in-culture predator training efforts have had limited success in improving survival of juvenile salmonids released into natural streams (see Thompson 1966, Kanayama 1968, Berejikian et al. 1999). The efficacy of anti-predator training programs in existing hatcheries will likely depend on their ability to significantly improve postrelease survival and be easily and inexpensively applied.

Numerous non-salmonid fish species communicate danger through chemical alarm substances localized in the skin (see Smith 1992 for a review). Such chemical alarm signaling has also been demonstrated in rainbow trout (Brown and Smith 1997, Brown and Smith 1998), and suggests the possibility that these chemical signals may be applied to condition “naïve” hatchery salmonids to recognize and avoid predators after release. The chemical substances that elicit fright responses by conspecifics are most commonly produced in epidermal cells. Mechanical damage to these cells caused by a predator attack releases the alarm substance, which warns nearby conspecifics of possible danger and elicits fright responses (Smith 1992). Some species, including rainbow trout, learn to associate the predator odor with the perceived danger, and subsequently respond to the predator odor by itself (Magurran 1989, Chivers and Smith 1994, Chivers et al. 1995, Brown and Smith 1998). This transfer of the fright response to a neutral stimulus (predator odor) is known as releaser-induced recognition learning (Suboski 1988). Predator detection, and ultimately survival, might be improved after salmon are released into natural streams if acquired predator recognition could be achieved by treating fish with paired alarm signals in the hatchery.

Berejikian et al. (1999) examined the effects of chemical anti-predator conditioning on anti-predator behavior of chinook salmon (*Oncorhynchus tshawytscha*). Hatchery-reared juvenile chinook salmon was exposed to extracts from conspecific tissue or to comparable stimuli from swordtails (*Xiphophorus helleri*). These “injured fish” stimuli were paired with water that contained the odor of predatory cutthroat trout (*O. clarki*). Chinook salmon receiving conspecific stimuli showed higher levels of several anti-predator behaviors compared to chinook salmon receiving swordtail extracts. When the two groups of chinook salmon were tested 2 days later with cutthroat stimulus alone, the chinook salmon that had originally received injured conspecific stimuli paired with cutthroat trout odor spent more time motionless (a primary predator avoidance response: Healey and Reinhardt 1995) than chinook salmon which had received swordtail stimuli and cutthroat trout odor.

Several questions remain as to whether chemical conditioning can be used effectively in a typical hatchery operation. For example, Berejikian et al. (1999) found that the response of the chinook juveniles to predator odor 10 days after receiving chinook extract paired with predator odor did not differ from controls. When chinook juveniles were treated in hatchery vessels then transported to the laboratory, no differences in response to predator odor were found between treated and control fish. In these experiments, it is possible that i) handling stress may have interfered with predator recognition, ii) fish were unable to recognize predator odor after transfer to a novel environment, or iii) acquired predator recognition lasts for only a short time (see Berejikian et al. 1999 for details). The suite of experiments conducted addressed the importance of these factors and tested new techniques (e.g., repeated application of paired alarm signals) to improve the efficacy of anti-predator conditioning and its application to hatcheries. The study was conducted to determine whether: 1) populations which evolved in sympatry and allopatry with northern pikeminnows possess innate predator recognition; 2) fright responses to predator odor can be increased by pairing conspecific extract with predator odor; 3) handling affects conditioned responses; 4) acquired predator recognition is retained after transport to a novel environment; and 5) vulnerability to live predators in a stream channel can be improved by chemical conditioning.

Methods and Materials

Study Populations

Juvenile chinook salmon were obtained from the Carson National Fish Hatchery (CNFH), Carson, WA, and the University of Washington Big Beef Creek Hatchery (BBCH), Kitsap Co., Washington. The CNFH population has a stream-type (i.e. yearling smolt) life history. Fish released from the CNFH are exposed significant predation by northern pikeminnows (*Ptychocheilus oregonensis*; Collis et al. 1995). The Big Beef Creek Hatchery population exhibits an ocean-type life history and was derived from a combination of several Puget Sound populations. A total of 518, two-month-old chinook fry were transported from CNFH to the NMFS Manchester Research Station on 8 February 2000 to be reared in tanks supplied with 10°C well water (for Experiment 1). Thirty-eight hundred eyed eggs were also obtained from the BBCH. After emergence, 518 fry were transported to the NMFS Manchester Research Station (MRS) on 21 January 2000. At the MRS, the CNFH and BBCH fish were reared in separate, identical 1.8-m diameter vessels and fed a commercial pellet diet several times daily, 6 days per week.

Northern pikeminnows (NP) were collected from the Columbia River and the Yakima River either by boat-mounted electroshocking or by hook-and-line. Sixty fish were transported to the MRS between 16 February 2000 and 28 April 2000, and held in two 6-m diameter vessels. Odors of consumed prey fish can chemically label the odor of predators and their feces (Mathis and Smith 1993bc; Brown et al. 1995). Therefore, the NP were fed non-fish diet of earthworms for 3 weeks to reduce the potential that alarm pheromones of prey fish eaten by NP prior to their capture would be detectable in the chemical odor of the NP and their feces during the experiments.

Chemical Stimuli

Skin extracts and predator odors were prepared as follows for use in the experiments. Both fresh and frozen water containing the odor of NP were produced. Frozen NP odor was produced by placing two NP (> 250 mm) in an aerated water bath (40-L) for 17 hours. The water was frozen in 45 mL plastic vials. Fresh NP odor was produced by placing the NP into the water bath by 1500 hours the day prior to the day of the trials and removed by 0800 hours (17 hours total) the following morning. This procedure was repeated each time that behavioral trials were run with fresh NP odor. The water was then transferred into 45 mL lots and used in observational trials between 2 and 5 hours after removal of the NP. Distilled water was used as a control in several of the experiments was not frozen.

Juvenile chinook salmon were used to generate the skin-muscle extract (CE) for experiments 2, 3, and 4. The salmon were killed with a blow to the head. A total of 60 cm² of skin and muscle tissue was added to 3,000 mL of distilled water, homogenized and filtered through a polyester filter floss. Extract from green swordtails was produced following the same procedure. Green swordtails are phylogenetically distant, allopatric, and possess no apparent alarm pheromone (Mathis and Smith 1993a). The extracts were frozen in 45 mL lots.

Experimental Apparatus

Behavioral assays were conducted in two indoor flumes, each measuring 9.0-m long by 1.5-m wide. Ten 170-L aquaria were positioned in each of the flumes and were surrounded by a fairly constant temperature (12.5 to 13.0° C) water bath. The back and sides of the aquaria were opaque. The clear side of each aquarium faced the outside walls of the flumes, and allowed unobstructed viewing of the fish in each aquarium. Each aquarium contained a 3-cm deep layer of 1.0- to 1.5-cm diameter gravel. Water was delivered at a rate of 6 L·min⁻¹ through a funnel connected to a poly-vinyl tube that terminated at mid-water depth at one end of each aquarium. Water exited each aquarium through a double siphon at the end opposite the water inflow. Water depth was maintained at 45 cm. Rulers fastened to the back wall of the aquaria provided a visual reference for dividing the vertical water column into lower, middle, and upper thirds. Illumination was provided by a bank of wide-spectrum fluorescent lights on a simulated natural photoperiod.

Experiment 1: Innate recognition and response to NP

This experiment was conducted to test the null hypotheses that 1) behavioral fright response to NP odor is the same for chinook juveniles from the CNFH and juveniles from the BBCH population, and that 2) freezing the water containing NP odor does not affect behavioral fright response in either population.

Trials began by placing a single fish (45.0-60.0 mm fork length) from either the CNFH or BBCH into each of 20 aquariums (day 1). The fish were each fed about twenty-five 1-mm diameter commercial feed pellets three times daily over the following

3 days. On day 4, the fish were each fed another 20 pellets between 0745 and 0830 hours to standardize stomach fullness. Behavioral trials consisted of 8-min pre-stimulus and 8-min post-stimulus observations. Twenty minutes prior to beginning pre-stimulus observations 0.5 mL of live *Daphnia* (strained) were added to 10 mL of water and introduced into an aquarium. At the end of the 8-min pre-stimulus observation, 45 mL of either frozen NP odor, fresh NP odor, or distilled water were introduced through the water inflow tube.

Behavioral frequencies and durations were quantified using event recording software (Observer v2.0, Noldus Information Technology b.v. University Business and Technology Center). Reduction in feeding rate, increased time spent motionless, and increased time spent near the substrate are common anti-predator responses of juvenile salmonids (Gotceitas and Godin 1993, Healey and Reinhardt 1995, Berejikian et al. 1999). Thus, during both pre- and post-stimulus observations, observers recorded: 1) the number of food strikes; 2) the number of darts; 3) the amount of time spent in the lower, middle, and upper thirds of the water column; 4) the amount of time spent motionless. Any occurrence in which a fish captured a *Daphnia* was counted as a food strike. A fish was considered to be in a motionless state if its head moved less than 1 cm in 2 seconds. Darts were defined as sudden, rapid movements, in which the fish traveled greater than 1 body length.

A total of 105 trials were conducted between 06 March and 22 March 2000. The difference between pre- and post-stimulus observations was calculated for the behavioral durations and frequencies. Data were log transformed to improve homogeneity of variances. Data are being analyzed by a two-factor ANOVA where chinook population (CNFH and BBCH) and stimulus (fresh NP, frozen NP, distilled water) were the main effects. A Bonferroni test will be used to test for pairwise among group differences ($\alpha = 0.05$). We expect a final analysis to be complete by 31 January 2001.

Experiment 2: Releaser-induced predator recognition and effects of handling

This experiment was conducted to test the null hypotheses that 1) simultaneous exposure to predator odor and conspecific skin extract does not result in acquired predator recognition (i.e., learning), and that 2) 'handling' does not affect acquired predator recognition. Big Beef Creek Hatchery chinook salmon were used as subjects for this experiment and were sampled from the group held at the MRS. Acclimation and feeding protocols followed those in Experiment 1. Behavioral observation trials consisting of 8-min pre-stimulus and 8-min post-stimulus observations began on 12 April 2000. Twenty minutes prior to beginning pre-stimulus observations 0.8 mL of live *Daphnia* (strained) were added to 10 mL of water and introduced into an aquarium. At the end of the 8-min pre-stimulus observation, 45 mL of either CE or swordtail extract (SE), each combined with 45 mL of NP water were introduced through the water inflow tube. Observation techniques followed those described for Experiment 1.

Two days after introducing the paired stimuli, the same fish were tested for their response to NP odor alone. These trials were conducted in the same manner as the paired-stimulus trials except that only NP odor was introduced after the 8-minute pre-

stimulus observations. To simulate the transport of hatchery reared fish prior to release, half of the fish treated with CE + NP odor were removed from their aquarium, loaded into a truck-mounted fish transport tank, and driven for a 1-hour duration. The fish were returned to their original aquarium approximately 3 hours prior to testing their behavioral response to the NP odor alone. This created three treatments: i) CE + NP handled, ii) CE + NP not handled, and iii) SE + NP not handled (n = 19 per treatment).

The difference between pre- and poststimulus observations was calculated for the behavioral durations and frequencies. Data was transformed as needed using logarithmic transformation to minimize variance heterogeneity. Treatments will be compared using a one-way Analysis of Variance.

Experiment 3: Conditioning in rearing vessels, repeated treatments, and retention of conditioned response

The following experiment was conducted to test the null hypotheses that 1) there is no difference in acquired predator recognition of fish from tanks treated with i) a distilled water control, ii) one application of CE + NP odor, or iii) two applications of CE + NP odor, and 2) time between treatment with paired alarm signals and exposure to predator odor does not affect fright responses to predator odor.

Eleven hundred chinook fry (80.0 to 100.0 mm fork length) were transferred from the BBCH to the MRS on 12 May 2000 and placed into six circular tanks (183 per tank). Beginning on 18 May 2000 (day 1) two replicate tanks (tanks 1 and 2) were treated with 125 mL each of CE and frozen NP odor. Four tanks (3, 4, 5, and 6) were treated with 250 mL of distilled water control. On day 2 tanks 1, 2, 3, and 4 were treated with 125 mL each of CE and frozen NP odor and tanks 5 and 6 were treated with 250 mL of distilled water control. An observer positioned above the tanks recorded general behavior patterns of the fish in each tank prior to, during, and after the stimuli were introduced.

On day 3, fish were removed from each vessel (three or four fish from each of the six tanks; 20 total) and placed individually into each of the 20 aquariums. Feeding and acclimation protocols followed those described for experiment 1. On day 5, fish were tested for their response to NP odor alone. Trials consisted of 8-min pre-stimulus and 8-min post-stimulus observations. Twenty minutes prior to beginning pre-stimulus observations 1.4 mL of live *Daphnia* (strained) were added to 10 mL of water and introduced into each aquarium. At the end of the 8-min pre-stimulus observation, 45 mL of NP water were introduced through the water inflow tube. Observation techniques followed those outlined for Experiment 1. Upon completion of the observational trials, fish were removed and another 20 fish (three or four from each rearing vessel) were stocked into the aquaria. The process of stocking, acclimation, testing, removal, and restocking was repeated for a total of 60 trials (20 per treatment). Thus, fish from each treatment were evaluated 3, 5, and 7 days after the final treatment (on day 2). Table 1 describes the time-sequence of treatment and testing for experiment 3. Data were analyzed by a repeated measures ANOVA.

Table 1. Design of experiment 3, showing the time sequence in which the three treatments (two applications of CE + NP, one application of CE + NP, and control) were applied, the days on which fish were introduced (i.e., stocked) into the aquariums for observation and the days in which the fish were observed. Fish stocked on day 3 were tested on day 5, those stocked on day 5 were tested on day 7, etc. After testing on a given day, the aquariums were drained, refilled and restocked.

Tanks	Treatment		Day 3	Day 5	Day 7	Day 9	N
	Day 1	Day 2					
1 & 2	CE ¹ + NP ¹	CE + NP	Stock (6)	Test (6) Stock (8)	Test (8) Stock (6)	Test (6)	20
3 & 4	Distilled	CE + NP	Stock (6)	Test (6) Stock (8)	Test (8) Stock (6)	Test (6)	20
5 & 6	Distilled	Distilled	Stock (6)	Test (8) Stock (6)	Test (6) Stock (6)	Test (6)	20
Trials conducted				20	20	20	60

1/CE = chinook extract; NP = northern pikeminnow odor

2/Numbers in parentheses indicate the sample size per pair of tanks. Equal numbers of fish were stocked from each of the two tanks within a pair.

Experiment 4: Vulnerability to NP in a quasi-natural stream channel

The following experiment was conducted to test the null hypothesis that there is no difference in predator avoidance ability among fish from tanks treated with: i) a distilled water control, ii) one application of CE + NP odor, and iii) two applications of CE + NP odor. The experiment included exposure of fish from the three treatments to live NP in a quasi-natural stream channel. Chinook salmon for this experiment were sampled from the same tanks and on the same days described in Experiment 3.

The predation assays were conducted in a 45-m long by 6-m wide outdoor stream channel (3% gradient) located at the MRS. Well water was supplied at $80 \text{ L} \cdot \text{min}^{-1}$ and recirculated at a flow of approximately $5,100 \text{ L} \cdot \text{min}^{-1}$. Water temperature was maintained between 13.0 and 15.0°C over the course of the study. Eight replicate, 5.0-m long by 6.0-m wide sections were created in the stream channel, each bisected longitudinally by a solid wooden divider. The substrate was composed of 3- to 5-cm diameter gravel, which was graded to create as similar a depth and velocity profile as possible in the 16 single sections. Abundant aquatic insects (Family: *Chironomidae*) populate the channel, so no artificial feeding was necessary. Chironomid larvae, pupae, and adults were continuously available to the chinook juveniles throughout the study. One denuded, commercially farmed Douglas-fir tree (1.8-m long by 0.9-m maximum diameter) was partially submerged in each of the 16 single sections to provide complex submerged structure, overhead cover, and complex flow patterns. One side of a wooden box (0.6-m by 0.6-m by 0.5-m) was fitted with a plastic 4-cm square mesh screen and submerged into a downstream corner of each stream section. Juvenile salmon were introduced through a funnel emptying into an opening in the top of the box. Once introduced, the salmon could exit the box through the screen; however, the screen excluded NP from entering the box. An underwater viewing chamber positioned alongside the entire length of one of the 8 sections allowed for continuous (day and night) observations of predator and prey behavior.

Three predators (NP) were stocked into each of the 16 sections of the channel 10 days prior to introducing the juvenile salmon. On day 5, forty fish from each treatment (20 fish per tank) were introduced into each of 5 stream channel sections to create independent predation evaluations, such that the fish from one treatment would not be affected by the behavior of fish from the other treatments. PIT tag identifications were recorded prior to introducing the fish to the stream channel so the size and treatment applied to each fish could be determined when surviving fish were recovered from the stream channel. After 1 day of exposure to NP in the stream channel, the remaining chinook salmon were removed and enumerated. A second release of chinook salmon into the stream channel was performed on day nine, followed by removal of survivors on day 10. The proportion of fish eaten in each stream channel section trial was the response variable. Data will be arcsin transformed, and will be analyzed by a repeated measures ANOVA. Tanks will be treated as experimental units of replication, and test day (i.e., 5, 7) treated as the repeated measure. We expect a final analysis to be complete by 28 February 2001.

Results

Results have been reported in the following peer-reviewed publication:

Berejikian, B. A., Tezak, E. P. and LaRae, A. L. 2003. Innate and enhanced predator recognition in hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*). Env. Biol. Fish. (Accepted for publication).

A copy of this paper will be provided to BPA upon publication.

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Section 6

**SOCIAL BEHAVIOR AND COMPETITIVE ABILITY OF JUVENILE
STEELHEAD GROWN IN ENRICHED AND CONVENTIONAL
ENVIRONMENTS AND THEIR COMPETITIVE IMPACTS ON NATURALLY
REARED FISH
(PROGRESS REPORT FOR THE PERIOD MARCH 1, 2001 - FEB 29, 2002).**

by

**Stephen C. Riley, Barry A. Berejikian, Jeff A. Atkins¹, E. Paul Tezak, Anita L.
LaRae¹ And Eric Kummerow¹**

National Marine Fisheries Service
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Manchester Research Station
P.O. Box 130

Manchester, WA 98353

¹ Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon, 97027

Introduction

Millions of juvenile salmonids are released from hatcheries in the Columbia River each year to mitigate for lost habitat and support sustainable fisheries for Pacific salmon and steelhead (*Oncorhynchus spp.*). A small proportion of hatchery programs are designed to enhance, or 'supplement', salmonid populations, particularly those that are listed as threatened or endangered under the Endangered Species Act. The ultimate goal of hatchery supplementation programs is to restore wild salmonid stocks to a self-sustaining state. There are currently a number of supplementation programs underway in the Columbia basin (BPA 1992; Waples et al. in press), but there is little evidence that any have been successful (Winton and Hilborn 1994; IMST 2000, Waples et al. in press).

Two potential reasons for the lack of success of supplementation programs are high post-release mortality of hatchery fish (Maynard et al. 1995; Olla et al. 1998; Brown and Laland 2001) and negative ecological interactions between hatchery-reared and wild fish in streams (Nickelson et al. 1986; Flagg et al. 1995). Hatchery fish may use different habitats than wild fish and may suffer reduced survival as a result (Mason et al. 1967; Dickson and MacCrimmon 1984). Hatchery-reared salmonids are often more aggressive than wild conspecifics (Fenderson et al. 1968; Swain and Riddell 1990; Mesa 1991), and it has been suggested that energetic expenditures associated with increased aggression may contribute to the low survival observed for most stocked hatchery fish (Bachman 1984; Deverill et al. 1999).

Increased aggression by hatchery fish might also result in negative effects to wild fish resident in streams where hatchery fish are released, which might undermine the success of a supplementation program. Although it is widely recognized that the release of hatchery-reared fish into streams may pose risks to wild salmonid populations (Pearsons and Hopley 1999), there is currently insufficient information to allow a quantitative determination of the potential impacts on wild salmonids (ISAB 2001). Competition from hatchery fish has been suggested as a mechanism contributing to the decline in abundance of natural salmonid populations (Nickelson et al. 1986; Flagg et al. 1995). Hatchery fish may disrupt natural social patterns in streams through competitive interactions (Bachman 1984; Nielsen 1994; McMichael et al. 1999), and may affect the abundance, growth, and survival of natural salmonids (Fresh 1997).

It has been suggested that changes in hatchery rearing practices may be necessary to produce fish suitable for supplementation (Stickney 1994). Modifications to rearing density or water velocity have sometimes been shown to increase the performance or survival of hatchery salmonids (Cresswell and Williams 1983; Martin and Wertheimer 1989), but results are equivocal (Maynard et al. 1995; McDonald et al. 1998). Other experiments have shown that structural modifications to hatchery rearing environments may increase the survival of juvenile chinook salmon released into streams (Maynard et al. 1996), and may affect the agonistic behavior, dominance, growth, and space use of steelhead fry in novel experimental environments (Berejikian et al. 2000; 2001).

If hatchery rearing environments affect the dominance and agonistic behavior of hatchery-reared salmonids, they might also be expected to alter any effects that hatchery-

reared fish may have on wild fish. This experiment was conducted to determine whether the dominance, feeding rate, agonistic interaction rate, or space use of naturally-reared steelhead fry were differentially affected by the presence of steelhead fry reared in conventional or structurally enriched hatchery environments.

Methods

Study population

Eggs were obtained from artificially spawned steelhead from the Skookumchuck River, a tributary of the Chehalis River in western Washington. This hatchery population was derived from the local wild population and spawning protocols have continued to incorporate naturally produced steelhead into the broodstock each year. Eyed eggs were transported from their initial incubation site (Bingham Creek Hatchery, Mason County, Washington) to the University of Washington's Big Beef Creek Research Station (Kitsap County, Washington) in May 2001 for final incubation in constant 10°C well water.

Rearing treatments

Six hundred and fifty steelhead fry were stocked into each of six 1.8-m diameter circular tanks (water volume = 1,520 L; flow = 28 L·min⁻¹) in May 2001. Three enriched (EN) tanks contained the tops of two Douglas fir (*Pseudotsuga menziesii*) trees that were submerged to provide in-water structure. A double layer of brown and green camouflage netting hung on a circular PVC frame provided approximately 60% overhead shade cover. Each tank was outfitted with an underwater food-delivery system, which delivered food at the mid-water depth at two locations on opposite sides of the tanks (see Berejikian et al. 2000 for photographs of the tanks). Three conventional (CO) tanks contained no overhead cover or in-water structure other than a center standpipe, and received 28 L·min⁻¹ of water from above the water surface. Fish in the CO tanks were hand-fed by scattering the food across the surface of the water. Fish in all tanks were fed with equal frequency.

At the same time emergent fry were stocked into the rearing tanks, 1,950 fry were stocked into a natural stream channel, a screened-off section of a side channel of Big Beef Creek, Kitsap County, Washington. The 35-m long channel received 0.05 m³/s water flow from springs and the main channel of Big Beef Creek, which was augmented with 0.025 m³/s well water. Red alder (*Alnus rubra*), western cedar (*Thuja plicata*), and salmonberry (*Rubus spectabilis*) comprised the main canopy and streamside vegetation. Woody debris (12 denuded Douglas fir trees) was added to provide structure and cover from avian and terrestrial predators. Few fish predators were present. Steelhead fry were dependent on natural food produced within the channel. Fish density in the stream channel was reduced as fish were removed for use in the experiments.

Feeding and agonistic behavior

Experimental trials were conducted in two 10-m long by 1.5-m wide flumes previously described by Berejikian et al. (2000; 2001). Screens were placed

perpendicular to the flow in each flume to produce five 1.5-m long by 1.5-m wide sections in each flume. Each flume received 30 L/min of 12°C well water recirculated at a flow of approximately 1,700 L/min by 2-horsepower submersible pumps. Water depth was maintained at approximately 20 cm. Light was provided by wide-spectrum fluorescent lights on a photoperiod of 14 hours light/10 hours dark. The side walls of the flumes consist of double-paned glass, which allowed complete viewing of all fish in each section.

The substrate of each flume section consisted of a sheet of fiberboard marked with a 10 cm grid to facilitate the estimation of territory size by overhead video cameras positioned and activated from a remote location. Two velocity refuges were provided in each section by placing two 9 cm high by 10 cm wide pieces of aluminum on the substrate, one 40 cm from the upstream screen and 10 cm from the center, and another 80 cm from the upstream screen and 50 cm from the center, both on the left side of the section. Thawed frozen bloodworms were introduced into each section at a rate of two per minute during observation periods; this averages to approximately 0.642 g/m^2 of food per hour, which is within the range of food abundance observed within juvenile steelhead territories in a natural stream (Keeley and McPhail 1998). The worms entered each section through a single PVC tube that was located in the center of the section at the upstream barrier and positioned at a 45 degree angle to the left such that fish which positioned themselves in the upstream-most position on the left side of the section had first access to food.

This experiment was conducted using two densities of steelhead fry. In the low density trials, two fry (one natural fry and one conventional, enriched, or natural fry; total density = 0.9 fry/m^2) were matched for body weight (less than 7.5% difference) and simultaneously introduced into each section of the flume. Four fry (two natural and two conventional, enriched, or natural fry; total density = 1.8 fry/m^2) were similarly matched for body weight and introduced into each section for the high density trials; hereafter we refer to these rearing treatment combinations as CN, EN, and NN, respectively. Fish were allowed to acclimate in the flume for approximately 44 hours with minimal feeding (an average of one bloodworm every 2 hours). Fry used in this experiment ranged from 48 to 82 mm in length and from 1.08 to 6.02 grams in weight; fish size increased over the duration of the experiment, but treatments were balanced over time.

After acclimation, the number of food items captured and the number of attacks (nips, charges, and chases) and threats (lateral and frontal displays) were recorded for each fish simultaneously in the section for 10 min by two observers. It was not necessary to mark steelhead fry because naturally- and hatchery-reared fry differed in color, and observers were capable of keeping track of individual fish during the course of observations. The order of observation was from downstream to upstream in each flume, and treatments and densities were balanced among flume sections and among observation dates. All observations took place between 8 AM and 1 PM. A total of 120 trials (20 replicates of three treatments at two densities) were conducted between 8 Aug – 26 September 2001.

Space use

Fish in each flume section were recorded by a remotely-operated overhead video camera during the 10-minute periods when feeding and agonistic interactions were observed. Videotapes were played back at a later date and the position of each fish was recorded from the video tapes (as x and y coordinates on the grid that made up the cell substrate) at ten second intervals. These coordinates were used to produce 95% confidence ellipses using SAS. Space use for each fish was estimated as the area of the ellipse, and space overlap was estimated as the sum of overlapping areas of the ellipses. The mean crosswise (x) and upstream (y) positions of each fish within the flume section were also estimated from these coordinates.

Growth of natural and hatchery-reared fish

A growth experiment was conducted in a 45-m long by 6-m wide outdoor stream channel previously described by Berejikian et al. (2000; 2001). The sides and bottom of the channel are constructed of concrete at a constant 3% gradient. Well water was supplied at $80 \cdot \text{L} \cdot \text{min}^{-1}$, and recirculated by three submersible 2-horsepower pumps at a flow of approximately $5,100 \cdot \text{L} \cdot \text{min}^{-1}$. Water temperature ranged from 11.2 - 15.0°C throughout the experiment, with a diurnal fluctuation of approximately 2°C. A wooden barrier divided the stream along its entire length; the channel was further divided by seven wire mesh (3.0-mm opening) screens set on top of weirs situated across the channel, perpendicular to the flow. This configuration created 16 replicate 5.0 m long by 3.0 m wide sections in the channel.

The channel substrate consisted of 3 to 5 cm diameter gravel graded to create similar depth and velocity profiles among the 16 sections. Algal growth on the substrate and sidewalls supported abundant aquatic insect populations (primarily *Chironomidae*), so no artificial feeding was necessary. No piscine predators were introduced, and a single layer of 2.5-cm square mesh netting placed over the entire channel excluded avian predators. An underwater viewing chamber positioned alongside the entire length of one of the 16 sections allowed for general observations of steelhead behavior.

The growth experiment was designed to test the null hypotheses that a) the growth of naturally reared fish did not differ when they were stocked with enriched or conventionally-reared fish, b) fry reared in enriched tanks grew at the same rate as conventionally reared fish, and c) the increase in total fry biomass was the same for sections containing enriched or conventional fry.

Eight of the 16 sections simultaneously received 20 fry reared in enriched tanks (six or seven from each of the 3 rearing tanks) and 20 stream-reared fry; the other 8 sections simultaneously received 20 conventionally-reared fry (six or seven from each of the 3 tanks) and 20 stream-reared fry. Treatments were randomized among sections.

All fish were measured (FL), weighed (nearest 0.01 g), and stocked into the channel on 16 August 2001. In each section, either hatchery (enriched or conventional) or natural fry were marked by removal of the adipose fin; natural fish were adipose

clipped in half of the sections and hatchery fish were clipped in the other half. Fish were removed by seining 32 days later (18 September 2001), and were identified to treatment by adipose clip, and weighed and measured as above.

Instantaneous growth rates (change in wet weight (g)•d⁻¹) were calculated as:

$$\text{IGR} = (\log_e w_2 - \log_e w_1) \cdot \Delta t^{-1}$$

where w_1 is the mean initial weight of fry within a stream channel section, w_2 is the mean final weight within a section, and Δt is the duration in days between stocking and removal of fry.

Data Analysis

To determine the effects of density and rearing treatment on dominance, we used a log-linear model with three dependent variables – density, rearing treatment combination (EN or CN), and fish type (natural or hatchery). For comparisons of the proportion of active fish, the proportion of fish feeding, and the proportion of food eaten among rearing treatment combinations (CN, EN, and NN) and between densities we performed two-way analysis of variance (ANOVA) on arcsine square-root transformed proportions. Comparisons of feeding, agonistic behavior, and space use among rearing treatment combinations and densities were conducted using two-way ANOVA (with rearing treatment combination and density as main effects). Comparisons of feeding, agonistic behavior and space use between hatchery- and naturally-reared fish were made using three-way ANOVA, with rearing treatment combination, density, and fish type (natural or hatchery) as main effects.

Instantaneous growth data were analyzed by a single factor ANOVA with rearing treatment (enriched vs. conventional) as the main effect. Total biomass data were analyzed by ANCOVA with rearing treatment as the main effect and initial total biomass as the covariate.

Results

All data have been analysed, and a final report including Results and Discussion are being prepared.

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Section 7

**SOCIAL BEHAVIOR AND COMPETITIVE ABILITY OF JUVENILE STEELHEAD
GROWN IN ENRICHED AND CONVENTIONAL ENVIRONMENTS AND THEIR
COMPETITIVE IMPACTS ON NATURALLY REARED FISH
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by

**Stephen C. Riley, Barry A. Berejikian, Julie A. Scheurer, Christopher P. Tatara, Anita L.
LaRae¹, Robert Endicott¹, Eric Kummerow¹ Jeff A. Atkins¹ and, E. Paul Tezak**

National Marine Fisheries Service
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Manchester Research Station
P.O. Box 130

Manchester, WA 98353

¹ Pacific States Marine Fisheries Commission
45 Southeast 82nd Drive (Suite 100)
Gladstone, Oregon, 97027

Introduction

It is widely recognized that artificial propagation programs for Pacific salmonids may pose risks to wild salmonid populations, but there is currently insufficient information to allow a quantitative determination of the potential impacts (ISAB 2001). The National Research Council (1996), the Independent Scientific Group (1996), and the Independent Scientific Advisory Board (1998) have identified ecological interactions between hatchery and wild salmonids, including competition and predation, as important factors that may negatively affect wild salmonid populations. The NMFS FCRPS Biological Opinion (NMFS 2001) calls for research to mitigate ecological risks of hatcheries to wild populations. Salmonid populations listed as Threatened and Endangered under the ESA face ecological risks from hatchery programs operated for harvest augmentation or mitigation (production hatcheries) and programs operated for maintenance and recovery of listed populations (conservation hatcheries).

Competition from hatchery fish may be one of several factors causing declines in wild salmonid populations (Nickelson et al. 1986; Flagg et al. 1995, 2000). Hatchery fish can disrupt natural social patterns in streams through competitive interactions (Bachman 1984; Nielsen 1994; McMichael et al. 1999), and hatchery fish may affect the abundance, growth and survival of natural salmonids (Hillman and Mullan 1989; Fresh 1997). Although there is evidence that hatchery fish may affect wild salmonid production, replicated experiments that are explicitly designed to test the effects of competitive interactions from hatchery-released steelhead on the behavior, growth and survival of wild salmonids have not been performed.

Salmonids reared in enriched (e.g., NATURES) hatchery environments may have higher survival rates in natural streams than conventionally-reared fish (Maynard et al. 1996). Steelhead fry grown in enriched rearing environments engage in significantly more threat displays than conventionally-reared fish and are more likely to socially dominate conspecific competitors (Berejikian et al. 2000, 2001). Steelhead fry reared in enriched environments might therefore have greater competitive effects on wild salmonids than conventionally-reared fish if they are more likely to threaten and socially dominate wild competitors. Because the majority of work on agonistic behavior and social dominance of hatchery and wild salmonids has been conducted in laboratories, the relevance of the results to natural streams is unknown. In this study, we released steelhead fry from conventional and enriched rearing environments into two streams to determine how rearing environment may affect the habitat use, feeding, movement, agonistic behavior, group size, use of cover, growth, and condition of hatchery-reared steelhead in natural streams.

Predation of wild juvenile salmonids by hatchery-released fish has been identified as a potentially important source of mortality on wild salmonids (Flagg et al. 2000). Several studies have observed predation of wild juveniles by hatchery fish (Sholes and Hallock 1979; Cannamela 1993; Hawkins and Tipping 1999), but few quantitative estimates of the overall impact on wild salmonid populations are available. Moreover, few studies have attempted to quantify any sublethal effects that piscivorous hatchery fish may have on wild salmonids. For

example, it is widely recognized that the presence of predators may reduce the foraging activity of prey (Lima and Dill 1990), and modeling results suggest that a reduction in foraging time due to the presence of predators may result in recruitment limitation for juvenile fishes (Walters and Juanes 1993). Quantitative estimates of direct mortality and sublethal effects on wild salmonids resulting from predation by hatchery fish are required for the development of models to estimate the ecological risks to wild salmonid populations associated with hatchery fish releases.

Some steelhead released from hatcheries have been observed to remain in the stream as ‘residuals’ after the majority of fish have migrated as smolts (Martin et al. 1993; Jonasson et al. 1995; McMichael and Pearsons 2001). Residual steelhead have been observed to prey on wild salmonid fry (S.C. Riley, unpublished data), but we know of no published studies that have attempted to determine the extent of this predation or to quantify the potential ecological risks (including sublethal effects) to wild salmonids. In this study, we quantified the effects of the presence of residual steelhead on the feeding, behavior, growth and survival of naturally- and hatchery-reared steelhead fry in a laboratory flume and an outdoor stream channel.

Methods

Spawning, incubation and rearing.

Twenty-five thousand eyed eggs were obtained from artificially spawned steelhead from the Skookumchuck River (Mason County, WA). Eggs were initially incubated at the WDFW Bingham Creek Hatchery. At the eyed stage of development, the embryos were transported to the National Marine Fisheries Service’s (NMFS) Big Beef Creek Research Station for final incubation in constant 10°C well water. One thousand, six hundred and sixty-seven emergent fry were stocked into each of twelve 1.8-m diameter circular tanks near the end of May 2002. Six enriched tanks contained two submerged commercially grown Douglas fir trees to provide structure, and a double layer of brown and green camouflage netting hung on an aluminum frame provided approximately 60% overhead shade cover. Each enriched tank was outfitted with an underwater feed-delivery system which distributed food from automatic feeders equally through two 2.5-cm diameter nylon tubes near mid-water depth (four food outlets per tank). Six “conventional” tanks contained no overhead cover or inwater structure. Fish in the conventional tanks were fed by automatic feeders which scattered the food across the surface of the water. All tanks received a constant flow of 40 l/min of well water. Fish were fed a 2% ration every half hour during daylight hours until they grew to 1g, 1.5% ration every daylight hour up to 1.5g, and 1.5% ration every 2 daylight hours thereafter. Fluorescent lights controlled by a 24-hour timer mimicked the natural photoperiod.

At the same time emergent fry were stocked into the rearing tanks, 5,000 emergent fry were stocked into a screened-off section of a side channel of Big Beef Creek. The 35-m long section receives 0.06 m³/min water flow from springs and the main channel of Big Beef Creek. Red alder (*Alnus rubra*), western cedar (*Thuja plicata*), and salmonberry (*Rubus spectabilis*) comprise the main canopy and streamside vegetation. Large and small woody debris was added to provide structure and cover from avian and terrestrial predators; no fish predators were

present. Steelhead fry fed on the substantial natural food produced within the channel. Steelhead fry from the channel are referred to as 'naturally-reared' fry.

Yearling steelhead used as predators in subtasks 3 and 4 were 2001 brood year Skookumchuck stock that were transferred to the Big Beef Creek Research Station in May 2001. These fish were reared at the Big Beef Creek facility in six 1.8m enriched (see above) circular tanks from May 2001 until they were used in the experiments.

Determine the effects of stocking steelhead fry reared in conventional and enriched environments on inter- and intraspecific interactions with natural fry in a natural stream

This experiment was conducted to determine how rearing environment may affect the habitat use, feeding, movement, agonistic behavior, group size, use of cover, growth, and condition of hatchery-reared steelhead in natural streams. This experiment was conducted on Eleven and Twelve creeks, two second order tributaries of the Skookumchuck River in the Chehalis River drainage in southwestern Washington. These creeks are situated on property owned by the Weyerhaeuser Corporation upstream of the Skookumchuck Reservoir. Fish passage is blocked by the Skookumchuck dam, but a 'trap-and-haul' operation run by PacifiCorp and the Washington Department of Fish and Wildlife (WDFW) transplants approximately 450 adult steelhead trout (*Oncorhynchus mykiss*) above the reservoir each spring to spawn naturally. Adult steelhead are also collected below the dam for broodstock for a hatchery supplementation project (WDFW Bingham Creek Hatchery, Satsop River, Mason County, Washington State.). In addition to steelhead, cutthroat trout (*O. clarki*) and sculpins (*Cottus* spp.) are present in Eleven and Twelve creeks.

The study reaches selected on each stream made up the majority of stream habitat downstream of impassible barriers to fish migration that were located less than 600 m upstream of the mouth of each creek. Study reaches were bounded by a weir on the downstream end and a natural barrier to migration on the upstream end. The total lengths of the study reaches were 483 and 560 m on Eleven and Twelve creeks, respectively. Each reach was divided by a weir into two study sections of approximately equal length. Weirs were constructed of panels (0.6m x 1.5m) framed with PVC pipe and covered in 6mm wire mesh. Panels were attached to steel fence posts driven deep into the stream banks and substrate. A wire mesh skirt extending upstream along the bottom edge of each weir panel was buried in gravel and cobble to prevent fish passage beneath the weirs.

Stream habitat in all study sections was surveyed on 6 August 2002. Habitat was quantified within discrete habitat units (pools or riffles), which were defined as those that were at least as long as they were wide; pools had a minimum residual depth of 0.3m. Length, wetted width, average and maximum depths, percent substrate by category (sand, gravel, cobble, boulder, and bedrock), and the number of pieces of large and small wood (>2m long; large >30cm diameter; small 10-30cm diameter) were quantified for each unit. Stream discharge was estimated once per week on each stream throughout the study.

Four thousand NATURES and conventional hatchery fish were stocked into Eleven and Twelve creeks on 13 August, 2002 (Table 1); one thousand fish of a single treatment were stocked into each study section. Five hundred fish from each release group were weighed and measured prior to release for growth estimates. All hatchery fish were double marked with adipose and pelvic fin clips (to distinguish them from wild fish) and were released at the mid-point of each study section. NATURES steelhead were stocked into the upstream section of Twelve Creek and the downstream section of Eleven Creek; conventionally-reared steelhead were stocked into the downstream section of Twelve Creek and the upstream section of Eleven Creek. An additional 250 hatchery steelhead of the same rearing treatment were introduced into each section on 27 August 2002 to determine the efficacy of the weirs at preventing fish movement into adjoining sections. These fry were given unique fin clips to distinguish them from the previously stocked hatchery steelhead and were also stocked at the midpoint of each section (Table 1). Fish from the second stocking were not distinguishable from the earlier stocked fish during snorkel surveys.

Table 1. Number, rearing treatment, and fin clips of hatchery steelhead stocked into the upstream and downstream sections of Eleven and Twelve creeks, summer 2002.

		Eleven Creek	Twelve Creek
Downstream Section	Initial (n=1000 each) Treatment Fin clips	NATURES adipose/left pelvic	Conventional Adipose/right pelvic
	Supplemental (n=500 each) Treatment Fin clips	NATURES adipose	Conventional Adipose
Upstream Section	Initial (n=1000 each) Treatment Fin clips	Conventional adipose/left pelvic	NATURES adipose/right pelvic
	Supplemental (n=500 each) Treatment Fin clips	Conventional adipose/right pelvic	NATURES adipose/left pelvic

Fish in the study sections were observed by snorkeling over a five-week period to quantify the movement, distribution, habitat preference, group size, use of cover, and position in water column of steelhead fry stocked into the two streams. Divers proceeded in an upstream direction and data were recorded by habitat unit, study section, and stream. Observations were conducted in each study section before hatchery fish were released to determine the distribution and abundance of wild fish, three times during the week hatchery fish were released (immediately after stocking, and again one and three days post-release), and twice weekly for four weeks thereafter. Divers recorded the number of fish, origin (wild or hatchery), size, proximity to vegetative cover, and position in water column for each individual or group observed. Fish were considered part of a group if they were within two body lengths of each

other. Likewise, fish were considered associated with vegetative cover if they were within two body lengths of submerged vegetation or directly beneath vegetation overhanging the channel. The position of each fish or group of fish in the water column was categorized as either the upper or lower half. Data were recorded underwater onto plastic wrist slates. For each habitat unit, the percentage of the total area snorkeled was estimated visually. The same two divers conducted all counts and snorkeled each habitat unit an equal number of times to minimize observer bias. Snorkel surveys took place between 10:00 and 17:00 (with the exception of a single night survey of each section) and were conducted only when visibility was greater than 3 m.

The weirs that separated the study sections on each stream were fit with trap boxes constructed of PVC pipe and plastic storage tubs to monitor fish movement into and out of the study sections and to estimate upstream and downstream dispersal rates. Two traps were fit to the mid-reach weirs to monitor upstream and downstream movement; a single trap was installed on the downstream weir in each reach to track downstream movement and emigration from the study area only. Traps were checked every other day for the five weeks of the study. All salmonids captured were anaesthetized, identified, measured, and examined for existing marks. Sculpins and other taxa were released after first capture on the opposite side of the weir. Salmonids captured for the first time were given a small caudal fin clip and released on the side of the weir they originated from. Wild salmonids captured a second time were released on the opposite side of the weir. Hatchery recaptures were removed from the study area to avoid mixing treatments.

Frequencies of feeding and agonistic behavior were estimated for wild and hatchery fish by underwater observation in Eleven and Twelve creeks. Divers entered each study section from the downstream end and moved upstream slowly to minimize fish disturbance. Once a group of two or more fish was encountered, the observer estimated the number of fish of each species and rearing group within an area of 0.5 m^2 . Divers observed all fish within this area and recorded the number of aggressive acts and feeding strikes by hatchery and wild fish during a five-minute period. Feeding strikes included both attempts and successful strikes; aggressive acts included all types of threats and attacks. Divers also recorded the number of potential salmonid predators (yearling or older) present within the area. A total of over 300 individual behavioral observations were collected on five occasions between 20 August and 10 September 2002.

We estimated the territory size of wild and hatchery-reared steelhead fry in the study streams by underwater observation. Divers located individual steelhead fry and observed them for 10 minutes to estimate territory size. Divers drew a map of the stream substrate on a slate and recorded the locations of the focal point, feeding forays, and agonistic interactions. After observations were complete, divers measured distances to landmarks on the substrate maps and used these distances to scale the maps. Distances from the focal point to the locations of agonistic interactions and feeding attempts were used to estimate territory size. Territory size estimates were conducted for 40 wild and hatchery steelhead in Eleven and Twelve creeks on 4 and 11 September 2002.

At the end of the study, we sampled each study section by three-pass electrofishing to estimate the growth, survival, and dispersal of hatchery fish and the distribution and abundance of wild fish. Each study section was divided into 25-m long subsections, and the capture location of each fish was recorded as being within these subsections and within the habitat units defined during the habitat survey. All fish captured were anaesthetized, identified, measured (fork length to the nearest mm), weighed (nearest 0.1 gram), examined for marks, and returned to the habitat units where they were captured. The four study sections were sampled on four consecutive days (16 – 19 September 2002).

Determine the effects of the presence of hatchery steelhead residuals from enriched and conventional rearing environments on the foraging time and survival of naturally-reared juvenile salmonids.

This experiment was conducted to determine whether the feeding, aggression, and shelter use by naturally-reared steelhead fry were differentially affected by the presence of steelhead fry reared in conventional and enriched environments in the presence and absence of predators. Trials were conducted in two 10-m long by 1.5-m wide flumes. Screens were placed perpendicular to the flow in each flume to produce ten 0.75-m long by 0.75-m wide sections in each flume. Each flume received 30 L/min of 12°C well water recirculated at a flow of approximately 1,700 L/min by 2-horsepower submersible pumps. Water depth was maintained at 24 cm, and light was provided by wide-spectrum fluorescent lights on a photoperiod of 16 hours light to 8 hours dark. The sides of the flumes consist of double-paned glass, which allowed complete viewing of all fish in each section.

The substrate of each flume section consisted of a sheet of white fiberboard, and a velocity refuge was provided by placing a 9 cm high by 10 cm wide velocity barrier on the substrate of the section. A structure constructed of black PVC was placed on the substrate of each section against the viewing glass such that four separate shelters were visible to observers. These shelters were accessible to fry via four holes in each shelter and served as simulated hyporheic refuges for fry. Thawed frozen bloodworms were introduced into each section at a rate of 5-20 worms per 10 minute observation period. The worms entered each section through a single plastic tube that was positioned at the head of each section such that fish in the upstream-most position had first access to food.

This experiment was conducted using three densities of steelhead fry. In the low density trials, two fry (one natural fry and one conventional, enriched, or natural fry; total density = 3.6 fry/m²) were matched for body weight (less than 7.5% difference) and simultaneously introduced into each section of the flume. Four (two natural and two conventional, enriched, or natural fry; total density = 7.1 fry/m²) or eight (four natural and four conventional, enriched, or natural fry; total density = 14.2 fry/m²) fry were similarly matched for body weight and introduced into each section for the high density trials. A single yearling steelhead (hereafter predator) was simultaneously introduced into each section of one of the flumes, i.e., half of the sections observed contained predators. Fish were allowed to acclimate in the flume for approximately 44 hours with minimal feeding. After acclimation, the number of food items captured and the

number of attacks (nips, charges, chases) and threats (lateral and frontal displays) by natural and hatchery fry were recorded in the section for 10 min by two observers; these observations were repeated the following day. The use of shelters and position in the water column (upper or lower half) of hatchery- and naturally-reared fry were estimated by scan observations that were undertaken three times daily for three days during the course of each replicate. The allocation of treatments and fish densities was balanced over flume sections and over time. A total of 180 replicates (10 per treatment at each of three densities, with and without predators) were conducted between 10 July – 19 September 2002.

Determine the effects of steelhead post-smolt residual steelhead from enriched and conventional environments on survival of naturally-reared steelhead fry in a semi-natural stream channel

This experiment was conducted to determine whether the growth and survival of naturally-reared steelhead was affected differentially by the introduction of fry from conventional and enriched rearing treatments in the presence of predators. This experiment was conducted in a 45-m long by 6-m wide outdoor stream channel. The sides and bottom of the channel are constructed of concrete at a constant 3% gradient. Well water was supplied at 80 L/min and recirculated by four submersible, 2-horsepower pumps at a flow of approximately 6,800 L/min. A 5-horsepower pump continuously delivered 350 L/min of water from the stream through a chiller to maintain temperature between 11.0 and 15.0°C. A total of 16 replicate 5.0-m long by 3.0-m wide sections were created in the stream channel by a wooden barrier which divides the stream along its entire length into two side-by-side channels, and by seven wire mesh (3.0-mm opening) screens set on top of weirs situated across the channel perpendicular to the flow. The substrate consisted of 3- to 5-cm diameter gravel graded to create similar depth and velocity profiles among the 16 sections. Algal growth in the channel supports abundant aquatic insect populations (primarily *Chironomidae*), and no artificial feeding was necessary; high densities of recirculating chironomids were reduced by installing a fine-mesh drift net in the headbox of the channel to produce natural densities of drifting prey.

Natural fry were stocked into the channel alone and in combination with steelhead fry reared in enriched and conventional environments at three densities (0.9, 1.8, and 3.6 fry/m²) on 30 July 2002. Two yearling steelhead reared in enriched environments were simultaneously stocked into all sections as predators. All fish were removed from the sections on 1 October 2002 and the survival of natural juveniles was assessed. All fish were measured (fork length, nearest mm) and weighed (nearest 0.1 gram) on introduction and removal for analysis of growth and condition.

Results

Data from all experiments are currently being entered into computer databases or are under analysis. Analysis of all data is expected to be completed by October 2003. Writing and preparation of peer-reviewed journal articles based on these data are expected to be completed by December 2003.

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